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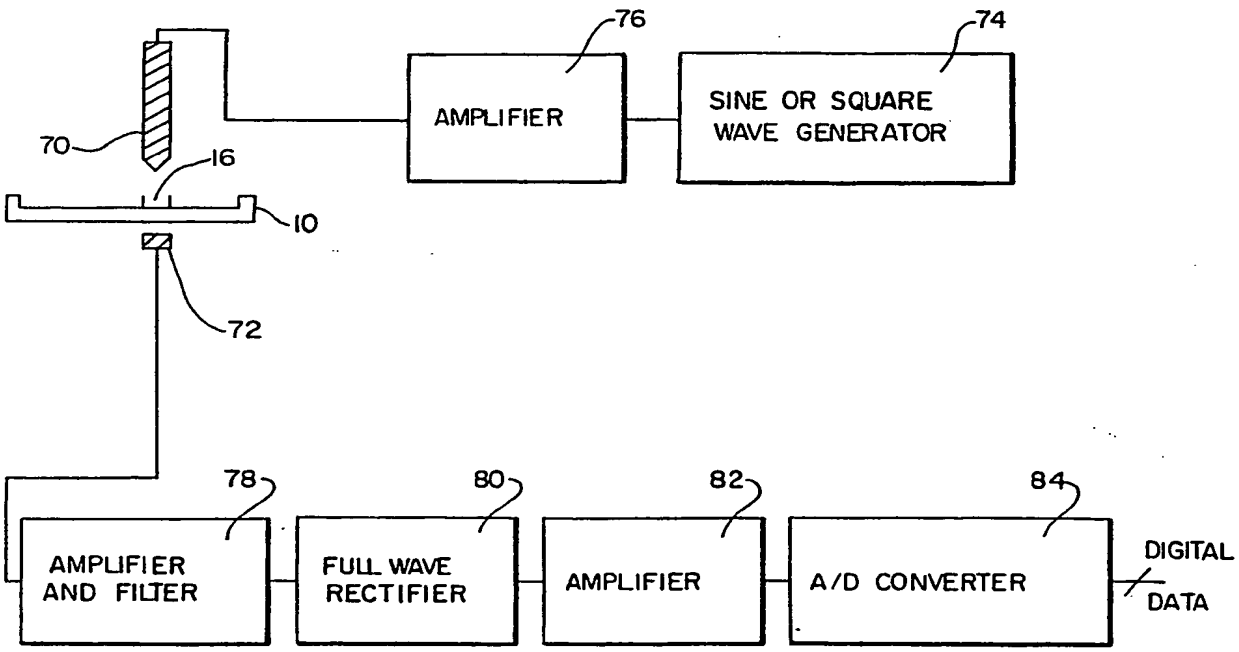

(57) Abstract

A liquid dispensing mechanism (34) is provided which includes a dispensing probe having a tip (70) for dispensing liquid, a pump for collectively discharging fluid from the probe tip (70) in measured quantities such as droplets. The dispensing probe has the capability to detect droplet formation and droplet separation from the probe (70) through use of a radio-frequency signal (74, 76) to the probe tip (70) and a conductive element (72) connected to amplifying and analyzing circuitry (78, 80, 82, 84) disposed below the dispensing probe (70) and a reaction well (16) which contains a receiving fluid. The conductive element (72) also detects when the probe has been inserted into the receiving fluid.

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LIQUID DISPENSING MECHANISM

BACKGROUND OF THE INVENTION

The present invention relates generally to an automated apparatus and method for performing assay testing on specimens, such as biological specimens. More specifically, the invention is directed to an automated apparatus and method for assay performing procedures to detect the compatibility of tissue or blood from a donor to a recipient.

Modern test procedures for determining or measuring the optical or electrochemical development of unknown specimens are used extensively in a number of medical testing procedures. In such tests, sample specimens are reacted with reagents and other substances. Such known procedures involve a variety of different assay steps but typically rely on detection and measurement of optical changes in a sample or label during the assay procedure. For example, a number of well known procedures use single or multi-wavelength fluorescence. These and other immunoassay techniques are known as Fluorescence Polarization Immunoassay (FPIA), solid phase agglutination, stained cellular morphology, enzyme Immunoassay (EIA), chemoluminescence and spectrophotometric assays.

Other currently used assay techniques are effected by exposing the resulting sample to either transillumination or reflectant illumination. These assay procedures involve detecting the intensity of colorization, detecting ratio of

multiple wavelengths of colorization, detecting the polarization in the sample, determining the size and quantity of specific cells at certain wavelengths, the general cell morphology or other optical characteristics of the results. The data from these procedures is then processed in a known manner to obtain the concentration or ratio of the component (or components) of interest. These techniques, however, have not been completely accepted and usually manual analysis is also performed as a check or verification.

One assay procedure of particular interest is a procedure known as Human Leukocyte Antigen (HLA) typing. This procedure is employed in matching tissue, body organs or blood from a donor to a recipient. In this HLA procedure, lymphocytes in samples containing human cells are first isolated and then reacted with different antisera. The cell-serum mixture is then incubated with a complement. One or more stains are added to the mixture, with one of the stains staining dead cells. The reactions are then evaluated by calculating the ratio of dead cells (lysed cells) to live cells. The calculations are performed by using a microscope and estimating the ratio. This ratio is converted into a "score" ranging from 1-8 by using a well-known value scale.

In this HLA procedure, as well as other assay procedures, paramagnetic particles are coated with an antibody. The paramagnetic particles are then mixed with a sample to be analyzed. The antibody on the paramagnetic particles binds to specific cells in the sample. These specific cellular components may then be separated from the other cells in the population which is being tested using magnetic separation techniques. Alternatively the cells may be separated by using a nylon wool column. After the cells have been reacted with the antibody, the sample mixture is subjected to a series of operations such as particle exposure, reagent exposure, incubation, and washing. The cells in the sample may also be stained with one or more chemical markers

as discussed above with respect to HLA assays. The sample is then analyzed. Typically, the sample will be analyzed manually by the technician. This manual analysis usually involves a visual analysis to determine the approximate percentage of the cells which have reacted with the antibody.

A significant shortcoming of these and other available assay techniques is that most of the steps in the procedure must be performed manually. For example, most of these procedures require manual preparation of the sample. Further, steps such as dispensing, mixing, washing, incubation, data collection, scoring and recording are also performed manually. Thus, most available assay techniques require a significant amount of human operator time.

As will be apparent to those skilled in the art, manual performance of these steps is also undesirable since it results in numerous opportunities for errors to occur. This is especially true for highly repetitive functions. The probability of errors is further amplified by the fact that many of these procedures require pipetting of very small volumes, i.e. usually of sub-microliter volumes. Further, scoring of thousands of reactions using a microscope and pencil also increase the probability of errors in the analysis.

A further drawback is the subjectivity which is permitted to the individual performing the test. This subjectivity may lead to inconsistent results, not only from assay to assay, but inconsistent analysis during the numerous repetitions in the same assay.

Although some available HLA assay devices automate individual steps, most of the steps in these devices are still performed manually. For example, U.S. Patent No. 4,318,866 (Kawahara et al.) discloses an apparatus for HLA typing which uses a phase-contrast microscope and an optical image to generate a signal which is detected by an electrical signal pickup unit. The image is then binarized and compared with

predetermined template patterns corresponding to reacted or non-reacted lymphocyte. Although the scoring of the results is automated, the preparation, incubation and washing of the sample must still be performed manually by the operator.

Further, this apparatus uses dedicated electronic hardware to score the HLA typing test. As discussed in more detail below, anomalies such as dirt or dust in the sample, scratches in the sample container, or unusually large cells would result in unreliable or erroneous results. In fact, without redesign for such possible variations, many human readable samples are unreadable by this apparatus. Variations in the procedures used by the operator preparing the samples may also lead to unreliable results without major redesign of the system. In summary, any expansion of the apparatus to score assays other than those that it was specifically designed for is difficult and costly, requiring major redesign of the hardware for each assay.

Another major disadvantage of available automated systems, such as the one disclosed in U.S. Patent No. 4,318,866, in that they are designed for a specific assay procedure (such as HLA typing). It is not possible to perform assays for which the instrument was not originally designed without a major redesign of the hardware and or software. Such major redesign is impracticable and thus the use of available instruments is limited to a single type of assay.

Precise dispensing of the sample in reaction wells is also critical for accurate assay results. In HLA typing the dispensing is usually performed manually. A certain manual dispensing operation may include dispensing sample volume of from 0.5 µl to 1.0 µl into a volume of 0.5 µl of a reagent which is covered by 2.5 µl of mineral oil. (The oil is used to prevent evaporation of the reagents.) In the alternative, larger volumes are necessary to reduce the effect of operator error. It will be appreciated that performing this dispensing step involves a significant amount of operator time, which

increases as the number of different reagents increases. In addition, increasing volumes increases cost as many of the reagents may be quite expensive. Further, the operator will usually insert the tip of the pipette below the bottom surface of the oil and into the reagent itself. In order to prevent carryover from one reaction site to the next, the operator will typically manually wipe the tip of the probe thus consuming more operator time and increasing the chances for erroneous results.

If automated assay apparatus and methods are to be used in HLA assay procedures, they must be capable of very precise monitoring of liquid levels and precise control of liquid dispensing mechanisms (such as a pipette). Precise dispensing mechanisms are particularly important in HLA typing since, as discussed above, very small volumes of liquids (sub-microliters) have to be dispensed and usually into a container which contains another liquid. Although some automated liquid dispensing systems are presently available, they are not completely suited for dispensing samples for assays such as HLA typing. Available automated liquid dispensing systems usually work by detecting the liquid level in a container and then determining the position of the dispensing probe relative to the liquid surface. This information is then used to determine when the probe tip is within the liquid in the sample container. After the liquid surface has been detected and it has been determined that the probe is in the fluid, fluid may be dispensed into or aspirated from the container. The precision of the liquid dispensing system will thus depend in part on the precision of the liquid level detection.

The limited potential for available liquid level detection and fluid dispensing systems in HLA assay typing is due to the fact that they typically use a capacitance method to detect the liquid surface as a pipetting probe moves towards the liquid in a sample container. Dispensing liquids

in volumes smaller than one microliter is complicated in such capacitive or conductance systems since the oil which covers the reagent has a low dielectric constant. The dielectric constant of oil is only two times greater than the dielectric constant of air rendering most capacitive detection methods unreliable for detection of the oil surface. Further, because of the high resistivity of the oil, available conductance methods cannot be used accurately.

Available capacitive type dispensing systems also do not have any means for determining when a droplet of the sample is formed on the dispensing probe or when a droplet of the sample has been separated or released from the probe tip. The ability to detect the occurrence of one or both of these events is important information which could be used to improve the accuracy and reliability of the dispensing system.

Therefore, it would be desirable to have a liquid level detection and liquid dispensing arrangement capable of detecting very small amounts of liquid (down to fractions of a microliter) and with the capability of detecting the level of a liquid having a low dielectric constant.

Another area of assay testing where significant improvements are necessary is in the area of image processing used for counting reactions. Although photomultiplier tubes have been previously used in some HLA readers, they are not without disadvantages and have not been readily accepted in the market. These readers use the photomultiplier tube as a fluorescent densitometer to measure the overall light output from the reaction site for each wavelength. This may be acceptable for ideal samples but produces critical errors if any contaminants, such as dirt, dust, or other interfering substances or other anomalies, such as scratches, are in the reaction site. The errors arise because this approach cannot determine the features in an object, such as shape or size of particulates in the reaction site. Therefore, there is a need for an instrument with the improved capability for the

discrimination of features within the field of view of the imaging device. Although higher magnification and selective mask techniques may be developed for the photomultiplier tube to yield the desired selectivity, the cost, reliability and throughput of such a device would make it impractical. In addition, such devices do not produce an image to which a human technician is accustomed and therefore that image could not be scored by the technician to confirm the instrument generated result.

Therefore, in view of the above, it is a primary object of the present invention to provide an apparatus and method for automatic processing of a qualitative, quantitative or morphological analysis of test specimens including serum, plasma or cellular components as well as other non-biological specimens.

It is another object of the present invention to provide an automated instrument for performing HLA typing, including automated cell separation, automated sample processing, and automated reading of the results.

It is a further object of the present invention to provide an apparatus and method for performing an assay on a disposable or reusable cartridge on which the specimen to be analyzed may be placed and which will be analyzed by an automated instrument.

It is another object of the present invention to provide an analytical instrument with a liquid dispensing and liquid level detection system which can control liquid dispensing of very small volumes, accurately determine liquid levels even in liquids with a relatively low dielectric constant, and determine droplet formation and separation.

It is another object of the present invention to provide a detection system which can detect the interface between liquids with different dielectric constants.

It is another object of the present invention to provide an analyzing instrument with powerful, cost-effective

and efficient image processing for automated sizing and counting of data.

It is yet another object of the present invention to provide an apparatus which is field upgradeable to perform different types of assays.

SUMMARY OF THE INVENTION

To achieve these and other objects, the present invention comprises an apparatus which automates the steps required in an assay procedure including cell separation, sample processing, dispensing and scoring of the assay results.

The apparatus performs the assays on a reaction cartridge having a plurality of reaction wells having different reagents disposed thereon. At least one well is provided in the reaction cartridge to receive a sample. The cartridge includes a well for containing particles adapted to bind to the sample and which have the capability of being separated from cells (such as paramagnetic particles) which do not bind to the separation particles, a well with at least one fluorophore adapted to bind to a specific type of cell in the sample is also provided. The cartridge includes a wash area adapted for washing a probe and reservoirs for retaining liquid and waste.

The apparatus of the present invention includes an optical or image forming arrangement is provided to detect images which indicate whether specific reactions have occurred in each of the reaction wells. The apparatus also includes a mechanism for dispensing and aspirating liquids including a mechanism for detecting liquid levels. The device further includes logic for analyzing the information received from the image forming arrangement and for processing the information to generate a visual indication of the assays being performed and their results. A microprocessor is provided to assist in

the operation of the device as well as in the image processing.

In another aspect of the invention, a particularly novel configuration for a cartridge which may be used in the apparatus and methods of the present invention is provided. The cartridge includes a plurality of reaction wells having different reagents disposed therein. The cartridge may also includes unit volumes of separation particles, a well adapted to receive a unit volume of the sample to be analyzed, and a well for storing a unit volume of dye (such as a fluorescent dye) which may be used in the analysis of the sample. The cartridge also preferably includes a well which may be used as a probe wash area.

In another aspect of the present invention, a particularly unique arrangement is provided for detecting multiple liquid levels and for dispensing fluids. The liquid level and dispensing mechanism includes a probe through which a fluid is dispensed. The system includes the ability to detect when a droplet has been formed by the probe and when the droplet has been separated from the probe. An oscillator provides a radio-frequency signal to the tip of the probe. A conductive element connected to amplifying and analyzing circuitry is disposed below the dispensing probe and the reaction well. The conductive element receives the radio-frequency signal from the probe and processes the signal to determine when the probe has reached the surface of a liquid in the well, when a droplet has been formed and detached from the probe, and when the probe is inserted into the liquid.

The inventive instrument is a random access, automated instrument system designed to perform HLA and PRA (Panel Creative Antibody) testing for transplant diagnostics. The instrument utilizes disposable or reuseable cartridges which incorporate sample well(s), reagent well(s), reaction well(s) and probe wash/waste well(s) into a single unit.

Although the number and configuration wells changes depending upon the assay the external size of the disposable is preferrably at 5.5"l. x 3.3"w. x 0.55"h. Both human readable and machine readable (barcode) labels can be affixed to the disposable for identification.

As illustrated in the Figures, the major sub-systems which comprise the instrument are the pipette robot, read robot, fluidics system, reader, load, unload and incubator stations. The instrument functions are controlled by an on-board PC based computer controller. The human interface and data management functions are accomplished by an external PC based Human Interface Workstation.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the invention. The objects and advantages of the invention may be obtained by means of the combinations particularly pointed out in the appended claims, including all equivalents.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is one embodiment of a cartridge of the present invention for holding reagents and samples to be analyzed.

FIGURE 2 is an embodiment of one of the reaction wells in the cartridge illustrated in Fig. 1 depicting reagents and a droplet in the well.

FIGURE 3 is a block diagram of an embodiment of the major components of the analyzing arrangement of the present invention.

FIGURE 4 is a schematic block diagram of a top view of an embodiment of the apparatus and method illustrated in Fig. 3.

FIGURE 5 is one embodiment of a three axis robot including a gripper which may be used for the pipette and image robots illustrated in Figs. 3 and 4.

FIGURE 6 is an embodiment of the gripper which may be used in the three axis robot illustrated in Fig. 5.

FIGURE 7 is a block diagram of an embodiment of the liquid level sensing arrangement of the present invention.

FIGURE 8 is a block diagram of the liquid level sensing and dispensing mechanism of the present invention.

FIGURE 9 is one embodiment of the amplifying circuits used for the liquid level sensing and dispensing mechanism illustrated in Figs. 7 and 8.

FIGURE 10 is an illustration of the output signal from the liquid level detection system of the present invention for a first dispensing procedure.

FIGURE 11 is one embodiment of a square wave oscillator which may be used in the liquid level sensing and dispensing arrangement illustrated in Figs. 7 and 8.

FIGURE 12 is schematic of one embodiment of the optical or image forming arrangement of the present invention.

FIGURE 13 is a schematic, in block diagram form, of an embodiment of the image processing arrangement of the present invention.

FIGURE 14 is an illustration of the output signal from the liquid level detection system of the present invention for a second dispensing procedure.

FIGURE 15 is front perspective view of the apparatus in accordance with the invention presented with the cover removed showing major components of the analyzing apparatus.

FIGURE 16 is a top view of the apparatus of Fig. 15 with the top cover removed.

FIGURE 17 is a side cross-sectional view of another embodiment of the optical arrangement of the present invention.

FIGURE 18 is a perspective view of another embodiment of a three axis robot including a gripper which may be used for the pipette and image robot illustrated in Figs. 3 and 4.

FIGURE 19 is an illustration for determining location of the well within an image for software determination of profiles AB, BC, CD, BC, CB, and DA.

FIGURE 20 presents the profiles for the image in Fig. 19 again utilizing the coordinates of Fig. 19. When a profile is analyzed individually, it may be represented by one of four possibilities:

Type 1 - all white pixels
Type 2 - all black pixels
Type 3 - one transition
Type 4 - two transitions

FIGURE 21 illustrates a grouping of different orientations of the well if the profiles have a 1-3-2-3 ordering.

FIGURE 22 shows the well orientations for a 4-2-2-2 ordering.

FIGURE 23 illustrates configurations of 1-1-3-3 ordering.

FIGURE 24 shows the images corresponding to a 2-2-3-3 ordering of the profiles.

FIGURE 25 is an illustration of the image in which the software has detected an extreme position of the well in the X-axis.

FIGURE 26 is an illustration of the results of the software controlled movement of the cartridge so the position of the edge of a well is centered in the frame.

FIGURE 27 is an illustration of the well being moved to locate the extreme position at a predetermined coordinate which will ensure that the well is centered in the frame.

FIGURE 28 shows a region of interest superposed on an image of the well and frame.

FIGURE 29 shows a typical histogram and threshold for the region of interest (ROI).

FIGURE 30 illustrates ideally a value of the threshold which would be set at location on the histogram.

FIGURE 31 illustrates a typical grey scale contour of two cells and also illustrates the two cells with a different threshold.

FIGURE 32 illustrates multiple thresholds calculated during the process of cell counting.

FIGURE 33 illustrates images A-E going from a strong negative, to weak negative, to weak positive, to positive and to strong positive reaction.

FIGURE 34 presents on top a row of graphs A-E illustrating process intensities with the X-axis being pixel position information and Y-axis grey scale intensity; the bottom row of graphs in Fig. 34 illustrate derivative of the intensity information and a method of classification and scoring of the information.

DETAILED DESCRIPTION OF THE INVENTION

System Architecture

Referring now to the drawings, Figure 1 illustrates a preferred embodiment of a test cartridge 10 which is used in the analysis of the specimens to be tested. In the embodiment illustrated in Figure 1, the cartridge 10 is particularly suited for HLA tissue typing. Although this and other embodiments which will be described are directed to HLA analysis, it will be readily apparent to those skilled in the art that the disclosed apparatus and methods may also be used with other assay procedures.

The tray or cartridge 10 includes two sample wells 11a and 11b. The second well may be used as a redundant

sample well which holds a sample for a second attempt at using the cartridge if the first sample does not provide satisfactory results. The sample cartridge 10 also includes a reagent well 12 which is used for storing paramagnetic particles and a fluorescent dye or fluorophore. The fluorescent dye may be, for example, of blue excitation and green emission wavelengths. A well 13 contains a complement reagent and a second fluorophore. The second fluorophore preferably excites at green and emits at red wavelengths. The cartridge 10 also includes a probe wash area is with a plurality of separate wash basins 14 (ten shown). The wash basins 14 drain off into the center of the probe wash area 15. A blotter 19 can be disposed in the center of the probe wash area. The blotter 19 absorbs excess fluid to prevent splashing or spilling during transport of the cartridge 10. Since the blotter 19 absorbs the waste fluid, it also facilitates the disposal of these wastes since they are now in solid form and may be disposed with the cartridge 10 itself. Preferably the blotter material is chosen to define a bi-axial transorb reservoir in the probe wash area 15. A suitable blotter material is a bonded cellulose acetate, such as is available from American Filtrona Co. (Richmond, VA).

As illustrated, the cartridge 10 includes a plurality of reaction wells 16. In the embodiment illustrated in Figure 1, the cartridge 10 includes 72 reaction wells on each side of the center area of the cartridge 10. Thus, in an embodiment a total of 144 or more reaction wells 16 are provided. The reaction cartridge 10 may also include blotter material 17 to absorb reaction and wash fluids. The blotter 17 is held in the cartridge 10 by means of pins 21 and ribs 23.

Thus, the cartridge 10 advantageously provides an arrangement where unit doses of the required reagents, dyes and separation particles and a well for a unit sample can be

provided. Additionally, this configuration permits the automation of the assay steps.

Referring now to Figure 2, a preferred configuration for the reaction wells 16 of the cartridge 10 is illustrated. Each of the reaction wells preferably has a 0.020 inch diameter bottom and a 0.090 inch top diameter and a height of 0.090 inches. The reaction wells may be formed on a cartridge made of mineral oil free, high-grade polystyrene by known techniques, such as injection molding. The inner surface of the reaction wells 16 is preferably plasma treated by known gas plasma (or gas ionization) treatment techniques, such as by the techniques disclosed in the article entitled "Treating Plastic Surfaces With Cold Gas Plasmas", P. Rose et al., Plastics Engr., Oct. 1, 1980, which is hereby incorporated by reference. In the embodiment which is illustrated in Figure 2, each reaction well 16 has 0.5 microliters of antisera covered with 2.0 microliters of an oil (such as mineral oil) to prevent evaporation. As will be recognized by those skilled in the art, the amount of oil may be varied in the well. For example, the well may contain 2.0 or 2.5 microliters of oil. Figure 2 also depicts a droplet 25 containing 0.5 microliters of sample which has been dispensed in the layer of oil 24.

Referring now to Figures 3 and 4, the major components of an embodiment of the apparatus of the present invention is illustrated in block diagram form. The apparatus includes a load area 30 and a stat load area 32. The stat load area 32 may be used to hold cartridges 10 with a higher priority than those in load area 30. Thus the cartridges 10 loaded into stat load area 32 will be processed first. The cartridge 10 illustrated in Figure 1 is inserted manually into either load area 30 or stat load area 32. Preferably the cartridge 10 includes a key 18 which is used to align or orient the cartridge 10 in the gripper arm of a robot (described in more detail below). A pipette robot 34

(discussed in more detail below) includes a gripper which grasps the cartridge 10 from the load or stat area and transports the cartridge 10 to an image capture area 42. The image capture area 42 may include means for taking an image of tray labeling information, such as barcode or optical character recognition (OCR) type information. This information may be used to determine the desired assay or assays for the particular cartridge which is to be analyzed, and to record any sample or patient identification information. The information may then be stored in a database for subsequent management tasks. The image robot can move the cartridge past the image capture area or a dedicated reader from the load area 30 or stat load area 32 so that the barcode can be read by the instrument.

The apparatus includes microcomputer and electronic circuitry 44 which will schedule the operations required to complete the desired assays after the assay requirements have been identified in a manner known in the art.

Preferably, the apparatus also includes a user interface 48 which may be used by the operator to manually enter information into the microcomputer memory or to download such information via a serial communications interface or read such information from a removable magnetic device.

As illustrated in Figure 4, the apparatus also includes a container for buffer 52, a power supply 41, a sample pump 50 and may optionally include a wash pump 54.

The apparatus or instrument illustrated in Figures 3 and 4 is more clearly shown as a working instrument in Figures 15 and 16. Figures 15 and 16 show the instrument with major components identified as in Figures 3 and 4. The front perspective view of Figure 15 and the top view of Figure 16 are both presented with the covers removed, thus allowing views of the components in actual working relationships rather than simple box diagram presentation as in Figures 3 and 4.

The pipette robot 34 and the image robot 40 may be

any suitable three axis robot. Figures 5 and 18 illustrate two different views of a presently used embodiment. The three axis robot comprises three stepper motors 201, 202, and 204 which cooperate with respective translating screws to move a gripper arm 56 to a desired position. A brief description of the movement assembly in the X-axis is given here. It will be recognized by those skilled in the art, that the movement in the Y-axis and Z-axis will be performed in a similar fashion.

The X-axis movement assembly comprises the stepper motor 204 which is connected to a translating screw 208 to provide translational motion of a platform 203 which supports the remaining robot assembly. Guide rails 210 and cooperating linear bearings 206 are provided to stabilize the translational movement in the X-direction. Switches 212 are provided to determine the position and to control translational movement in the X-direction.

In one embodiment, the normal working stroke of the X-axis and the Z-axis will be 7.75 inches while the working stroke in the Y-axis will be 9.25 inches. Each axis would preferably be capable of positioning with a minimum accuracy of +/- 0.003 inches over the entire length of travel. The assembled three axis robot will preferably be capable of positioning with a minimum accuracy of +/- 0.005 inches over the entire travel of each axis. A minimum resolution of 0.001 inches per 1.8 degree step input (200 steps/rev.)is preferable for each axis. Each axis will be driven by a 200 step/rev., 4 phase, 8 wire stepper motor. Each axis will be capable of translating at a maximum velocity of 5.0 inches/sec. and be capable of translational accelerations for each axis of 50.0 inches/sec./sec. and a maximum translational deceleration, for each axis, of 50.0 inches/ sec./sec. Each stepper motor is connected to its corresponding translating screw through a zero-backlash coupling of the helical spring type or by direct connection. The X-axis would have a position sensor at each end of travel, and the Y-axis and the Z-axis shall have a

position sensor at the motor end of travel.

Suitable three axis robots may also be available from commercial sources, for example one available as Model No. 105073P-20E from DAEDAL (Harrison City, PA).

As illustrated, three axis robots 34, 40 include a gripper arm 56. Referring to Figure 6, the gripper arm 56 includes a base member 58 which is attached to the respective robot. The base member 58 is in turn attached to an angled member 59 which is in turn attached to a jaw assembly.

The gripper jaw assembly includes a fixed jaw 60 and a spring-loaded jaw 62. The gripper arm 56 is configured such that the jaws 60 and 62 are disposed perpendicular to the axis of the base arm 58. Each of the gripping ends of jaws 60 and 62 is angled to facilitate the gripping of a cartridge 10.

Notches 64 and 66 are provided on gripper jaws 60 and 62, respectively. The notches 64 and 66 advantageously engage ribs 27 on the cartridge 10 to grip and align the cartridge 10.

During a gripping operation, the cartridge is centered between the gripper jaws 60 and 62. As the arm 56 is moved along the Y-axis toward the cartridge 10, the ribs 27 engage the inner surface of each of the gripper jaws 60 and 62, thereby slightly opening the spring-loaded gripper jaw 62. The gripper arm 56 is advanced in the Y-direction toward the cartridge 10 until the ribs 27 engage the notches 64 and 66. When the ribs 27 have moved into the notches 64 and 66, the spring-loaded gripper jaw 62 moves back to its unbiased position.

Advantageously, sensors 68 are provided to detect the alignment of spring-loaded gripper jaw 62. The sensors 68 will determine if the gripper jaw 62 is in the unbiased position when the cartridge 10 is inserted. This provides an arrangement to detect whether or not the cartridge 10 is properly positioned in the gripper arm before further processing. Suitable detectors are slotted optical switches

sold under Model No. OPB990P51 such as those available from OPTEK (Carlson, Texas).

In order to release the cartridge 10 from the gripping jaw assembly, the cartridge 10 includes a lip portion which extends downwardly from the gripper jaw assembly. The lip portion (not shown) may be, for example a lip extending downwardly from one side edge of the cartridge 10 such as the side indicated by arrow 20. This lip portion is adapted to engage a fixed ledge (not shown) as the gripper arm 56 is moved away from the cartridge 10 along the Y-axis, the ledge and lip portion cooperate to release the cartridge 10 from the gripper jaw assembly.

The carrier 10 is then transported to a closed loop pipette area 36 where aspirating, mixing, dispensing, washing and/or particle separation operations are performed based on prestored information concerning the assay (discussed in more detail below). The pipette area preferably includes a magnet which is positioned near to the side or below the reaction wells 16 during particle separation and washing procedures.

The image robot 40 or the pipette robot 34 then places the cartridge into an incubation transfer area 38. The cartridge 10 is held in the incubation area 38 for a predetermined incubation time period sufficient for the required reactions to occur. The incubation area 30 is preferably accessible from both the pipette robot 34 side of the device as well as from the side of an image robot 40. After the pipette robot 34 has moved a cartridge 10 into the incubation area 38 it is then free to begin processing another cartridge. Preferably, the robots 34 and 40 have random access capabilities to allow return of cartridge 10 from the incubation area 38 to the pipette area 36 or other work areas as many times as needed, and as dictated by the prestored requirements of each assay.

Once all pipetting and incubation area processing has been completed for a specific cartridge 10, the image robot 40

then grabs the cartridge from either the incubation/transfer area 38. The image robot 40 then transports the cartridge 10 to an image capture area 42 where image information is determined and converted into electrical information for further signal processing by the microcomputer and electronic circuitry 44 (described in more detailed below). Once all required images have been captured for a specific cartridge 10, the image robot 40 transports the cartridge 10 to an unload area 46.

The pipette robot 34 and the image robot 40 preferably operate independently of each other thereby allowing for parallel processing of the cartridges 10.

Although the instrument, as described, has been designed to run the HLA and PRA assays required for the transplant diagnostics market, it should be appreciated that the instrument is a very flexible automated pipettor and reader which could accommodate other test methodologies. Some of the benefits of the instrument are discussed below.

Many alternate disposable configurations may be accommodated. In a cartridge with an exterior size of 5.5"l. x 3.3"w. x 0.55"h., many sizes and combinations of sample wells, reaction wells, mixing wells, wash wells and reagent wells can be designed into a disposable. Practically the only limitation is that the disposable must be readable from the bottom and illuminated from either the top or bottom.

Assay protocols and procedures may be varied and mixed. That is any number of pipette steps, incubation steps and read steps can be accomplished in any order. The duration of the incubation steps may also be varied. The one limitation to mixing assay protocols is that throughput usually is adversely affected.

The range of pipetting volumes is wide. Testing to date has included 0.5 µl to over 50 µl per dispense. Due to the small diameter of the dispense probe (0.010"), which is required for the 0.5 µl dispenses, dispenses of 100 µl and up

require excessive amounts of time. This limitation can be overcome by replacing the dispense probe with a probe of the optimum diameter for the volumes being dispensed. The means of mixing on the instrument are through aspiration/dispensing of fluidics or mixing by movement of the disposable by the robot.

Disposables which have had manually prepared sample(s) placed in the appropriate well(s) are placed in the load station by the instrument operator. Up to ten disposables may be loaded into the load station at one time. The station actuates to separate the bottom disposable from the stack of disposables in the load station. By removing the bottom most disposable it is ensured that the disposables are run in the order in which they were placed into the instrument. The pipette robot moves to the load station and grasps the disposable in a gripper. The key on the disposable aligns the cartridge in the gripper. Sensors located in the gripper indicate to the computer controller that a disposable has been properly located in the gripper. The pipette robot then removes the disposable from the load station and moves it to the barcode reader.

The barcode reader is a fixed, LED type reader. The pipette robot moves the disposable to scan the barcode label, which is located on the end of the disposable, past the barcode reader. Upon successful reading of the barcode label, the computer controller identifies the disposable type and schedules the instrument activities required to process that disposable. Alternately, the imaging system can be utilized for this process.

The pipette robot is a three-axis linear robot which can move the disposable throughout the pipetting side of the instrument. The pipette robot can access the load, barcode reader, fluidics and incubator. If necessary, the pipette robot can access the reader though reading is typically

accomplished using the read robot. The pipette robot does not access the unload.

The fluidics system is capable of aspirating and dispensing fluids, performing magnetic separations, probe washing and liquid level sensing. In operation, the dispense probe is fixed and the pipette robot moves the disposable to the probe. An actuator is used to move a magnet into place for magnetic separations. Probe washing is accomplished in a probe wash well which is part of the disposable and all liquid waste is carried out of the instrument with the disposable. The liquid level sense is an RF (radio frequency) system using the dispense probe as a transmitter and having a receiving antenna disposed below the disposable and in line with the dispense probe. In some cases, the liquid level sense may also be utilized for dispense verification.

The incubator is heated and controlled to 34°C. +/- 2°C. Up to ten disposables may be stored in the incubator at any time. Either robot may place or retrieve a disposable in the incubator.

The read robot is identical to the pipette robot except that the gripper is reversed. The read robot can access the incubator, reader and unload. The read robot does not access the load, barcode reader or fluidics.

The reader on the instrument is essentially an inverted microscope having a CCD camera as a detector. A motorized objective turret allows the selection of one of four magnifications for the assay being read. Magnifications in the range of 1X to 10X have been tested. A quartz halogen lamp in conjunction with a fluorescence filter pack provides the foreground illumination used in the fluorescence assays and a LED provides background illumination for the agglutination assays. A motorized filter turret allows the selection of one of six filter packs for a fluorescence assay or no filter pack for an agglutination assay.

Each reaction well is automatically positioned and focused prior to image analysis. Image analysis for the HLA assays involves fluorescence imaging of stained white blood cells onto the CCD camera. Image analysis algorithms are used to count and size the cells in each image. For the agglutination assays, the agglutination pattern in the reaction well bottom is imaged onto the CCD camera. The result is then derived from the intensity profile across a diameter of the image.

Upon completion and result determination of an assay, the read robot moves the disposable to the unload. The unload then actuates to add the disposable to the bottom of the used disposable stack. Up to seventeen disposables may be stacked in the unload at any time. The load and unload capacities provide up to four hours of walk-away automation time.

One area that the instrument especially excels in is the ability to test one sample against many reagents. In the Class 1 HLA assay, a single sample is tested against up to 200 different antigens. The instrument layout is optimized to allow this type of testing to be accomplished in a minimum amount of time. This optimization could apply equally well to allergy testing, microbial susceptibility testing or any other type of testing which requires one sample to be tested against many reagents.

Image processing and data management are also strengths of the instrument. The use of CCD camera and image analysis allows reactions to be scored based on intensity, size, pattern or any combination thereof. Through the use of filters, or possibly the use of a color camera, color may also be used to score reactions. A standard PC as a human interface workstation provides an effective means of data collection, analysis and management. The human interface workstation may also serve as an interface to other lab instruments or to an LIS (Lab Information System).

PIPET ROBOT. The pipet robot is a three-axis X-Y-Z robot which is used to move trays through the pipetting section of the instrument. Each axis is driven by a stepper motor and a translating screw. Each axis also employs a home sensor to detect the home position of that axis. The X-axis is defined as left to right in the instrument and home for the X-axis is to the left. The Y-axis is defined as front to back in the instrument and home for the Y-axis is to the back. The Z-axis is defined as top to bottom in the instrument and home for the Z-axis is to the bottom.

Attached to the Z-axis of both the pipet and read robots is a passive gripper which is used to pick-up and hold trays. The gripper has two sensors which detect the presence and placement of a tray in the gripper. The no tray sensor detects that there is not a tray in the gripper. The misplaced tray sensor detects that a tray is in the gripper but misplaced.

In order to detect step loss, all long moves begin and end with all three axes in the home position. From the home position, the pipet robot can move to the load, the pipettor, the incubator and the reader.

READ ROBOT. The read robot is a three axis X-Y-Z robot which is used to move trays through the read section of the instrument. Each axis is driven by a stepper motor and a translating screw. Each axis also employs a home sensor to detect the home position of that axis. The X-axis is defined as left to right in the instrument and home for the Y-axis is to the back. The Z-axis is defined as top to bottom in the instrument and home for the Z-axis is to the bottom.

In order to detect step loss, all long moves begin and end with all three axes in the home position. From the home position, the read robot can move to the incubator, the reader and the unload.

READER. The reader is essentially an inverted microscope which images onto a CCD camera. Through the use of

an objective change wheel, a filter change wheel and two light sources, the reader can be configured as a fluorescence reader (for antigen assay) or an agglutination reader (for antibody test).

The objective change wheel and the filter change wheel are each driven by a stepper motor through a single set of gears. A 5.76:1 reduction is achieved by using a large SST gear at the perimeter of each wheel and a smaller urethane gear attached to the stepper motor. The center distance between the wheel and the motor is controlled to provide a slight interference between the gears, thus, producing a zero backlash drive.

The foreground illumination source is a quartz halogen lamp having an integral dichroic reflector. A condenser lens is used to focus the illumination at the object plane. A normally closed, solenoid operated, shutter blocks the foreground illumination when not in use so that the lamp may be left on continuously. A fan is used to cool this lamp and the hot air is ducted directly out of the instrument. The lamp is controlled by a constant voltage drive. No intensity control is provided.

The background illumination source is a LED. The LED is controlled by a constant voltage drive which is switched on when the LED is in use and off when not used.

For reading the antigen assay, the objective changer is moved to select the 10x objective and the filter changer is moved to select the first fluorescence filter pack (red). The LED is turned on to produce an image of the well which has a high contrast between the well sides and bottom. The auto-positioning and auto-focusing is now accomplished (described below). The LED is now turned off and the foreground illumination shutter is opened to image the dead cells (red) onto the camera and the first read image is captured. The filter changer is then moved to select the second fluorescence filter pack (green) and the live cells (green) are imaged onto

the camera and the second read image is captured. The foreground illumination shutter is closed to complete the reading of one well. This process is repeated for all wells.

For reading the antibody assay (in the HLA mode), the objective changer and the filter changer are moved to select the proper magnification and filter set (magnification varies from 1 - 4x depending upon well size). The backlight is turned on to image the agglutination pattern onto the camera. If needed, auto-positioning and auto-focusing is accomplished prior to capturing the read image. This is then repeated for all wells.

PIPETTOR. The pipettor holds a fixed pipet tip which also serves as a transmitting antenna for the liquid level sense system. A lower unit is actuated by a linear step motor. This lower unit consists of a receiving antenna which is spring loaded in the lower unit and a magnet arm for use in the magnetic separation step. A home sensor detects the home or down position of the lower unit.

Operation begins with the lower unit in the home (down) position. This allows a tray to be placed between the probe and the lower unit. The motor is then actuated to move the lower unit to the proper height for the operation desired.

PUMP. The pump is a dual syringe unit having a smaller sample syringe and a larger buffer syringe. The two syringes are connected to a single manifold having an inlet port and a discharge port. The inlet port is valve controlled. In the closed position the valve connects the buffer syringe to the manifold and closes the inlet port and in the open position the valve connects the buffer syringe to the buffer bottle and disconnects the buffer syringe from the manifold. The discharge port is connected directly to the dispense tip in the pipettor assembly.

The syringes are actuated by a rack and pinion drive which is driven, via belt, from a stepper motor. The valve is direct connected to a stepper motor.

Operation begins with the syringes in the home(up) position and the valve in the home (closed) position. To aspirate and dispense from the dispense tip, the valve remains closed and after the tip has been placed in the fluid the syringe is then moved upward (towards home) the appropriate distance for the dispense required.

To aspirate from the buffer bottle and then dispense out the dispense tip, the valve is first opened and then the buffer syringe is moved downward (away from home), thus, aspirating sample from the buffer bottle. The valve is then closed and the buffer syringe is then moved upward (towards home) the appropriate distance for the dispense required.

INCUBATOR. The incubator is a controlled temperature storage location for trays being incubated. Up to ten trays may be in the incubator at one time.

The conductive incubator is machined from a large block of aluminum. The high thermal conductivity of the aluminum minimizes the temperature differences from one area of the incubator to another. The large mass of the incubator provides a large thermal mass to minimize temperature fluctuations over time.

One of three heater configurations may be used. In the first configuration, two 3"x5" pad heaters are attached to the right and left sides of the incubator and the RTD (Resistive Thermal Device) sensor is located in the center. Fore the second configuration, the heater is a rod heater inserted vertically in the center of the incubator and the RTD sensor is surface mounted on the side. The third configuration uses a 2" wide heater wrapped around the top, bottom and right and left sides and a RTD sensor in the center.

Temperature control is provided by a stand-alone controller which may communicate with the instrument controller via serial link.

LOAD. The load station serves to accept a stack of up to ten trays from an operator and present one tray at a time, in a FIFO order, to the pipet robot. The load platform assembly is actuated by a linear step motor. A load platform assembly home sensor detects the home or up position of the load platform assembly and a load platform extended sensor detects the extended or down position. A tray-in-load sensor detects the presence of at least one tray in the load station. A door sensor detects whether the load/unload door is open or closed.

From one to ten trays may be stacked on the load platform assembly. As the load platform assembly is lowered, cam-actuated stops move in to hold all trays but the lowest, and continued lowering separates the bottom tray from the stack for pickup by the pipet robot. After the bottom tray is removed, the load platform assembly moves upward to hold the remaining trays as the cam-actuated stops move away.

Below the load station are two fixed STAT slots are for STAT trays. Trays placed in either of the STAT slots are to be processed before trays in the load station. Tray-in-STAT sensors (2) detect the presence of a tray in a STAT slot. The pipet robot may remove trays directly from a STAT slot.

Attached to the load station is a fixed, non-contact bar-code reader. After a tray has been removed from the load or STAT slot, the robot moves the tray to pass the bar-code label on the end of the tray by the bar-code reader, thus, reading the tray ID.

A solenoid actuated door lock is used to lock the load/unload door during operation of the load or unload station. This is to protect the operator from the load mechanism.

UNLOAD. The unload receives used trays from the read robot and stacks them into a stack of up to seventeen trays for removal by the operator. The unload platform assembly is actuated by a linear step motor. An unload platform home

sensor detects the home or up position of the unload platform assembly and an unload platform assembly extended sensor detects the extended or down position. An unload 75% full sensor detects the presence of at least thirteen trays in the unload station. An unload full sensor detects the presence of seventeen trays in the unload station. An unload door sensor detects whether the unload door is open or closed.

The unload remains in the home position until a used tray is ready to be unloaded from the instrument. As the read robot moves toward the unload with a used tray the unload platform assembly actuator moves the unload platform assembly from home to the extended position. Trays already in the unload are held in place above the unload platform by spring-loaded stops. The read robot places the tray on the unload platform and releases it or is disengaged from the tray via release features in the unload assembly. The unload platform assembly is then returned to the home position. As the unload platform assembly moves upward, the tray on the unload platform forces the spring-loaded stops open and adds the tray to the previous stack.

A solenoid actuated door lock is used to lock the load/unload door during operation of the load or unload station. This is to protect the operator from the unload mechanism.

LIQUID LEVEL SENSING AND LIQUID DISPENSING

As has been discussed above, the reaction wells 16 of the cartridge 10 contain micro-volumes of the antisera covered by a small micro-volume (approximately 2-3 µl) of oil. It is thus imperative that the liquid dispensing and liquid level sensing system used to dispense the samples to the reaction wells 16 be capable of detecting when the dispensing probe is inserted below the top surface of the oil (See Fig. 2).

In order to assure that a droplet of the sample (or other fluid being dispensed) has in fact been deposited into each reaction well 16, the apparatus preferably has the ability to detect when a droplet has been formed on the dispensing probe, when the formed droplet has separated from the dispensing probe, and when the dispensing probe has been inserted into either the oil or the serum. In the presently preferred mode of operation, the droplet is formed after the dispensing probe has been inserted into the oil or serum such that as the probe is pulled out of the liquid, the droplet of the sample will be "wiped off" of the dispensing probe. This technique combined with a closed loop system which uses the information regarding droplet formations and separations assures that a sample has in fact been deposited in each reaction well.

It will be, however, recognized by those skilled in this art that other modes of droplet formation and dispensing are possible. For example, the droplet may be formed on the dispensing probe in air before the dispensing probe is inserted into the liquid reagents.

An embodiment of the liquid dispensing system of the present invention is illustrated schematically in Figure 7. The liquid dispensing system includes a dispensing probe 70 for dispensing the liquid. As discussed above, the three axis robot can move the cartridge 10 in any of the X, Y, or Z directions by the use of stepper motors to position the dispensing probe 70 relative to a reaction well 16.

A sine or square wave generator (oscillator) 74 generates a radio-frequency (RF) signal which is amplified by an amplifier 76 and transmitted to the dispensing probe 70. A conductive element 72 is provided to receive the RF signals from the dispensing probe 70. The conductive element 72 is electrically connected to an amplifier 78. The amplifier 78 amplifies the signal received from the conductive element 72 for further processing as more fully described below. In

another embodiment, the conductive element 72 may be the magnet used in the particle separation process and working procedure described below.

The cartridge 10 is positioned such that a reaction well 16 is approximately centered under the dispensing probe 70. This positioning is achieved by initially training the robot. In a preferred embodiment, the dispensing probe 70 is about 3 mm above the bottom of the reaction well 16. In other embodiments the dispensing probe 70 may be positioned at other locations. For example, it may be disposed at the edge or rim of the reaction well such that the droplet will be dispensed on the surface of the wall of the well 16.

After the reaction well 16 is properly positioned, the monitoring of the signal from the oscillator 74 is initiated. The RF signal passes through the fluids inside the reaction well 16 and through the container and is received by the conductive element 72. The signal received by the conductive element 72 is amplified and filtered by an amplifier and filter 78. The signal is then rectified, preferably by a full wave rectifier 80, such that the output signal is a DC value corresponding to the amplitude of the received RF signal. The DC signal is then amplified by an amplifier 82 and converted to a digital signal by an analog to digital (A/D) converter 84.

Referring now to Figure 8, an embodiment of the control system for the liquid dispensing system is described. The DC signal which has been rectified and filtered may optionally be applied to a sample and hold circuit 86. The sample mode of the sample and hold circuit 86 occurs each time a pulse from a stepper motor control unit 88 is generated, thus providing synchronization between the DC signal value and the relative position of the sample cartridge 10. The DC signal is locked on the falling edge of the pulse from the stepper motor control unit 88 and a logical signal is sent to a digitizer 90. The digitizer 90 is preferably a twelve bit

ADC. The DC signal digitized value is then stored and analyzed by the microcomputer 44.

Alternatively, the system may be implemented without the sample and hold circuit 86 and the synchronization signal provided directly from the micro-computer 44.

The procedure described above of locking (from either stepper motor control or a microprocessor signal), digitizing and analyzing the DC signal with each pulse coming from the stepper motor control unit 88 continues until a sufficient difference between two consecutive stepper motor steps occurs. At this moment, the upward movement of the cartridge 10 may be stopped by a command sent to the stepper motor control unit 88. The relative position of the cartridge 10 is retrieved from the stepper motor control unit 88 by the microcomputer 44. If the relative position of the cartridge 10 is within a predetermined range (which has been stored in the memory of the microcomputer 44), then the process continues, otherwise an error condition will be reported.

After the liquid level has been identified as being within a predetermined range, the process continues with an additional movement of the cartridge 10 in the same upward direction for about 0.5 mm. During this movement, the DC signal is continuously sampled, digitized and analyzed to check for any unexpected conditions. At the end of this movement, the end of the dispensing probe 70 is reasonably assured to be inside of the oil 24 in the reaction well 16.

Next, a signal "M" from the microcomputer 44 is sent to disable the flow of pulses synchronizing with the vertical motion. The same signal "M" enables flow of the pulses synchronizing the DC signal values with a stepper motor driving and dispensing pump. A program command to run the stepper motor which drives the dispensing pump for a predetermined number of steps is issued and the DC signal value is again sampled, digitized and analyzed by the microcomputer 44. The process of dispensing a droplet

continues until an adequate increase in the DC signal is encountered, or the process is terminated if there is no increase or an unacceptable increases of the DC signal value.

After the droplet has been successfully produced or dispensed, a program command is sent to the stepper motor control unit 88 to move the cartridge 10 downward. During this movement, the DC signal value is sampled, digitized and analyzed by the microcomputer 44. When the tip of the dispensing probe 70 approaches the surface of the top liquid layer, such as the oil, the process of "wiping-off" of the droplet takes place and a rapid decrease in the DC signal value is observed to confirm that the droplet has actually been separated from the probe and dispensed into the reaction well 16.

The output signal V_{DC} from the liquid level detecting circuit of the present invention is illustrated in Figure 10. In the example, the probe 70 was inserted into a reaction well with reagent covered by a layer of oil and the droplet was formed in the liquid. The section of the curve from the origin to the voltage labeled "A" corresponds to the signal generated as the probe 70 approaches the upper surface of the oil. The section of the curve between the voltages labeled "A" and "B" corresponds to the signal generated as the probe 70 is advanced through the layer of oil towards the reagent. The section of the curve between the voltages labeled "B" and "C" corresponds to the formation of the droplet in the liquid. The section of the curve which decreases in slope after the voltage labeled "C" corresponds to the signal generated as the probe 70 is being withdrawn. The slope of the curve continues to decrease steadily until a time T_D when the droplet is released from the probe 70 and thus the slope of the curve decreases sharply.

It will be recognized that the signal illustrated in Figure 14 may optionally be differentiated such that peaks may

be generated and detected when there is a sharp change in slope. The differentiation may be performed by a suitable differentiating circuit or by the microcomputer 44.

Next, the RF amplifying circuit will be described. As shown in Figure 9, the amplifying circuit 100 is made up of two cascaded operational amplifiers 118 and 124. The positive input terminal of the operational amplifier 118 is connected to the conductive element 72 through resistor 111 and capacitor 110. The positive input terminal of the operational amplifier 118 is connected to a voltage dividing circuit formed of the resistors 112 and 113 keeping the output working point A at 1/2 of the supply voltage 109. The resistors 112, 113 and capacitor 110 acts as a high pass filter to reduce circuit sensitivity to low frequency signals. The negative input terminal of the operational amplifier 118 is connected to ground through the resistor 114 and capacitor 115 and is also connected to the output terminal through the resistor 116.

A decoupling capacitor 115 allows a high AC gain of the operational amplifier 118 with unity DC gain. The AC gain of the operational amplifier 118 is defined by resistors 116 and 114.

The output terminal of the operational amplifier 118 is connected to ground through resistor 117 and is connected to the input of the operational amplifier 124 through the capacitor 119 and resistor 120. The positive input terminal of the operational amplifier 124 is also connected to ground through resistor 121. The negative terminal of the operational amplifier 124 is connected to the ground through resistor 122 and is connected to the output terminal through the resistor 123. The gain of the operational amplifier 24 is defined by resistors 123 and 122.

Next, the full-wave rectifying and filtering circuit is explained. The rectifying circuit is connected to the output terminal of the operational amplifier 124 through

capacitor 125. In the illustrated embodiment of Figure 9 of the rectifying and filtering circuit 41, two operational amplifiers 137 and 138 are connected in a generally known configuration to various resistors, diodes and capacitor to produce a DC signal 139.

For negative signals from the amplifying circuit 100, the output of operational amplifier 137 is clamped to 0.7V by a diode 128 and disconnected from the negative terminal of the operational amplifier 138 by a diode 131. The operational amplifier 138 functions then as an inverter with input resistor 130 and feedback resistor 135 giving a positive signal at the output terminal of operational amplifier 138.

For positive signals from the amplifying circuit 100, operational amplifier 137 acts as an inverter with input resistor 126 and feedback resistor 132 and operational amplifier 138 operates as a summing inverter, again giving a positive output 139. When resistors 126, 129, 130 and 135 have the same value and resistor 132 is one-half the value of resistor 130, circuit 101 acts as a precision full-wave rectifier. The circuit 101 becomes an averaging filter when the time constant formed by resistor 135 and capacitor 134 is much longer than the maximum period of the input voltage which is to be averaged.

Referring now to Figure 11, one embodiment of a square wave oscillator circuit is illustrated. The square wave oscillator circuit comprise 5 resistors 215, 216 and 218, capacitors 217 and 220 and an operational amplifier 219. The oscillator which preferably operates at a 50 percent duty cycle, TTL levels is connected to the capacitor 217 and referenced to ground through resistor 215. A suitable oscillator is the function generator available from Wavetek as Model No. 145. The operational amplifier 219 amplifies the signal with a gain which is determined by the values of resistors 215 and 216. The output of amplifier 219 is AC coupled to the transmitting antenna through the capacitor 220.

Next, a description is given of another embodiment of apparatus and method according to the present invention. This embodiment is different from the other embodiments described above in that fluid dispensing takes place only when another fluid with a high dielectric constant has been detected. In the process described below, the two fluids have similar viscosities and thus the process will cause the two fluids to meld.

Again, the process starts with an upward movement of the cartridge 10 after a program command to move for a predetermined number of steps is issued. The upward movement continues until the oil surface is detected or end of the upward movement is detected. Once the dispensing probe 70 contacts the oil, the oil surface is detected and the program may issue a command to stop upward motion. At this time the relative position of the cartridge 10 is checked. If the relative position in the Z direction of the cartridge 10 is within a predetermined range (stored in a microcomputer memory) another program command to move cartridge 10 upward is issued. The number of steps to move upward is now equal to the predetermined value and upward movement continues until sufficient increase in the DC signal value between two consecutive stepper motor steps exists or when the end of the upward movement is detected. A rapid increase in the DC signal value manifests presence of a fluid with a dielectric constant greater than oil. The upward motion is then stopped. The dispensing process described above occurs. Figure 14 illustrates the signal from the detecting circuit for this embodiment. The signal at voltage level "A" represents the point where the oil surface is detected. The signal between voltages "B" and "C" at time T_1 represents the dispensing process when the probe touches the fluid on the bottom of the well. The curve between Times T_1 and T_2 represents a change in direction of the probe. At time T_2 the droplet is "wipe-off"

when the lower surface of the oil is reached. The signal does not decrease rapidly until the oil surface is encountered.

OPTICS

Referring now to Figure 12, one embodiment of the optical or imaging unit for the analyzing apparatus of the present invention is illustrated. The optical unit preferably includes a turret 177 containing at least two filter blocks 171. The turret 177 is rotated by a motor 175. Each of the filter blocks 171 has an excitation filter 170, emission filter 172 and a dichroic mirror 174. A light lamp 176 which is preferably a tungsten halogen lamp provides white light. The light is passed through a condenser 173 to condense the light before it passes through excitation filter 170 and is then reflected by a dichroic mirror 174 toward the cartridge 10. The light is then provided to the reaction wells 16 through a magnifying lens or objective 178. Preferably the magnifying objective is a 10X magnifying objective. The light is then reflected by objects within the reaction well 16. The light reflected from the sample well 16 passes through the objective and through the dichroic mirror 174 and then through the emission filter 172. The light transmitted through the emission filter 172 is then passed to a CCD element 185 of optical detector 180 where the light is processed as discussed in more detail below. As illustrated in Figure 12, the arrangement is similar to an inverted microscope reading through the bottom of each reaction well 16. As illustrated, the optical system also preferably includes an objective turret 179 adapted to hold at least two objectives 178. The optical system may also optically include a back light source 181 (discussed in more detail below).

In Figure 17, a side cross-sectional view of another embodiment of the optical unit is presented. The optical unit of Figure 17 is presented with reduced overall dimensions and shows additional elements not shown in Figure 12. For

example, a CCD camera 190; a filter block turret 230; and filter block turret motor 232 are shown in addition to the elements of Figure 12.

The filter block turrets 177 preferably have a range of rotation of a full 360°, with a radius allowing up to at least 6 filter packs, such as filter packs available from Nikon (Japan), to be rotated into the imaging position. The lens block or objective turret 179 preferably also has a 360° range of rotation with a radius allowing up to at least four standard microscope objectives, to be rotated into the imaging position. Each turret must be capable of positioning with a minimum of accuracy of +/- about 0.003 inches over the entire range of rotation. Each assembled optical module must also be capable of positioning with a minimum accuracy of +/- about 0.003 inches over the entire range of rotation of each turret.

As illustrated, the optical module preferably includes two direct/drive stepper motorized rotating platforms. Preferably each axis is driven by a 400 step/rev., four phase, eight-wire stepper motor, such as one available as Model No. PX24402DA, available from VEXTA (Tokyo, Japan).

Each of the filter block and lens turret sub-assemblies preferably has a position sensor at a "home" rotational location such that the filter pack and lenses are within + or - one step of the optimum optical path as measured by the peak intensity of light into the camera with a fluorescing test image. The sensors are preferably of the non-mechanical type such as a slotted optical switch available as Model No. OPB990P51 from OPTEK (Carlson, Texas).

For blue excitation-green emission, suitable filter packs are commercially available. A suitable filter pack, for example, is a B-2E Epi-fluorescence filter system available from Nikon (Japan). The dichroic mirror 174 preferably is positioned at 45° to the illuminator 176 and has a characteristic wavelength equal to about 510 nm. The excitation filter main wavelength preferably is 470 nm and the

band width is about 40 nm. The emission filter 172 has a spectro- transmission range from to 520 to 560 nm.

For the green excitation/red emission, the filter pack is also commercially available such as a G-2A Epi-fluorescence filter system available from Nikon(Japan). The dichroic mirror 174 is also positioned at 45° with respect to the lamp 176 and has a characteristic wavelength equal to about 480 nm. The excitation filter 170 has a main wavelength of about 535 nm and the band width is about 50 nm. The emission filter 172 spectro-transmission range is from 590 nm and up.

The magnifying objective 178 is also commercially available and may be, for example, a Nikon Plan 10 DL with a numerical aperture equal to 0.25 and a working distance of 5.2 nm. This objective lens with a large numerical aperture is desirable to enhance the brightness of fluorescence images.

Preferably, the lamp color temperature for the lamp 176 is at least 3000° K for the blue excitation. The lamp light output is preferably greater than 400 lumens.

It may also be advantageous to provide a neutral density filter (not shown) when using a combination of microscope objectives and relay lenses. In this embodiment, a 4.0 X perifocal magnifying objective may also be used in conjunction with the relay lenses and neutral density filter pack. A transmitting light source 181, such as an LED, may also be provided for use in reading agglutination assays.

The optical system will preferably include auto-focusing means for focusing the imaging. In one embodiment which is presently contemplated, an LED 181 is used to focus on the rim of the wells. Several auto-focusing algorithms for focusing with this technique are available in the art. For example one suitable algorithm is based on the "Threshold Gradient Magnitude Scheme". This algorithm is described in a paper entitled "Implementation of Automatic Focusing Algorithms for a Computer Vision System With Camera

Control", Schlag et al., Carnegie-Mellon University, August 15, 1983 (CMU-RI-TR-83-14), which is incorporated herein by reference.

Listed below in Table 1 are fluorophore excitation and emission wavelengths for suitable fluorophore which may be used in conjunction with the apparatus and methods of the present invention.

TABLE 1

Fluorophore Description	Wavelengths Excite	Emit
5(6) Carboxyfluorescein Diacetate - (Mixed isomers approx. 95% by HPLC) $C_{25}H_{16}O_9$ FW 460.4	490nm	520nm
Propidium Iodide - (approx. 95--98% by TLC) $C_{27}H_{34}N_4I_2$ FW 688.4	535nm (bound)*	602nm

* - exciting at bound emission frequency

IMAGE PROCESSING

As described above, the image processing unit used in the apparatus and method present invention determines the ratio of live to dead cells which have been stained with green and red stain in each reaction well 16. The score of the reaction in each well is based on the percentage of dead cells to the total number of cells. As currently practice in the art with manual scoring, the scoring is performed using a range of 1 to 8. A score of 1 indicates that mostly live (green) cells which did not react with the antisera are present. Conversely, a score of 8 indicates that mostly dead

cells which did react with the antisera and fluoresce red are present.

The size range of these cells of interest for HLA typing are from 6 to 12 microns in diameter,preferably with 100 to 300 cells per image. This translates into a minimum of 9 pixel areas per cell using a 512 x 484 resolution at 10X magnification and with bright fluorescence of some cells, a maximum of 81 pixel area for a single cell. The ratio of the average fluorescing cell image to the background mean is preferably at least 3 to 1.

As illustrated in Figure 13, in one embodiment, the image processing system includes a solid state charge coupled device (CCD) camera 190. The CCD camera 190 is coupled to a frame grabber 222. The frame grabber 222 preferably includes an onboard arithmetic and logic processing unit. A suitable frame grabber 222 is available from Coreco Montreal, (Montreal, Canada) as Model No. OC-300. Suitable software is also available from Coreco as a FG3 software package. The image procuring system is run by a PC computer with a display monitor 194. A suitable PC computer is an IBM AT Compatible 25 MHz 386 available from several commercial sources, such as Compaq. The system includes a monitor 192 for viewing the resulting images, such as a standard RS170 image monitor. A suitable monitor is available as Model No. VM-12016 from Hitachi Denshi, Ltd. (Woodberry, N.Y.). Advantageously, a digital signal processing (DSP) card 224 is expected to increase the system (discussed in more detail below). The DSP card 224 increases the throughput of the frame grabber 222 alone by a factor of 6. That is, using the frame grabber 222 alone, processing would take at least 4 seconds per image. With the DSP card 224 processing is expected to take less than 1/2 second.

Although data from the frame grabber 222 may be transferred to the DSP card 224 via the standard AT bus, this places a heavy load on the main microcomputer. Therefore the

DSP card 224 is preferably connected to the frame grabber 222 via a video bus (illustrated in the dotted line). This frees the main processor for other tasks.

A CCD camera which has proved suitable for use in the present invention is Model No. KP110 available from Hitachi Denshi, Ltd. (Woodberry, N.Y.).

The following describes several algorithms which may be used in the image processing stage of the present invention. In one embodiment, the FG3 software may be used to control both the range/offset and live/capture operations of the frame grabber. The range and offset may be, for example, typically set 16 and 32 respectively, shifting the value of zero 32/256 of full scale, and expanding 16/256 of the range to maximum intensity. It has been found that these values yield the highest contrast images with the least amount of noise. Once the image is focused as described above, the image is grabbed, then saved. From this point on, all processing may be done completely in computer RAM, with the results displayed on either an EGA or VGA screen.

A row normalization technique may be used to compensate for background light gradients. Each row of data (512 points) is summed, then divided by 512 and stored as the baseline for that row during thresholding. The total image normalization (row and column) at any point is the average of the row mean and the column mean at that point subtracted from the raw value at that point. To prevent any negative values, this may be implemented here by adding the (row) mean to a manually entered threshold.

A nearest neighbor filter convolution technique may be used to eliminate small "salt and pepper" noise from the image. For cells which are essentially round and at least 9 pixels in area, based on the 10X magnification, 1/2" size of the CCD element and a 6-12 M cell size, there is no interest in pixels that do not have neighboring pixels of greater intensity than a given threshold.

As will be recognized by those skilled in the art, there are many different kernels, or weighings, for this type of filtering. One approach which may be used will require any pixel to have at least one other pixel either above or below its position (either directly or diagonally) above the selected threshold, or the pixel value became zero. This eliminates all single pixel noise elements, and requires all "surviving" elements to be two dimensional.

A more general approach is typically a 3x3 kernel "K" mapped over a 3x3 area of the image "I", where each pixel is multiplied by the weighing in the corresponding value in the kernel. The results are summed, then divided by the total weighing.

Once the image has been normalized and filtered using the normalized value as the threshold, the algorithm preferably prompts for a manually selected threshold. This is determined by looking at the color of the background and comparing the brightest background color with the colorbar. Each color of the color bar has a value of 16 gray scale intensities in the original image. This value generally assures that all background is eliminated, at the possible expense of shrinking some weaker cells, depending on contrast and focus. Some experimentation may be needed to yield the best selection with minimum data loss. For example, the value 48 has been shown to work well with this approach.

Once all parameters have been selected, a "reverse fill" algorithm may be used to scan the image. This reverse fill algorithm scans from top left to right, and stop when the first non-zero pixel is detected. A counter is then preferably initialized and incremented as the search continues on that line until a (zero) background pixel is detected. The search then moves back to the first pixel, down one row, and searches to the left for the first background pixel. Since prior filtering has guaranteed that all elements are two-dimensional, this method is acceptable. When the

left-most non-zero pixel has been detected, the counter again is incremented until the right-most pixel has been reached. This process continues for each succeeding row until there are no further pixels below the last row checked.

This approach works well as long as the elements are more or less round, as are cells in the HLA assay. However, if cell clusters or other non-round elements are in the image, this version of the algorithm may have some shortcomings, especially in a horizontal large and small "dumbbell" type of element, where the large element on the left is properly sized except for a center row, but the right element will be split in half. Although these and other similar errors are possible, cell sizing and subsequent counting has been found to yield scoring comparable to human readings.

As each pixel is counted, its color is changed to the bright value by adding 8 to the selected binary color. Once the processing of the element is completed, its size is compared to the selected range. If the cell falls within that range, the process is repeated, changing the color to bright white (color-15). Raster scanning then continues looking for the start of the next element until the entire image has been searched.

Although this technique is suitable, it is very time consuming, and filling time is added to border detection time, causing even more overhead.

The above-described algorithms may be used on a system with either a VGA or EGA display card. If an EGA display card is used some modifications may be necessary since an EGA display card has 640 x 350 pixel by 16 color resolution and the images grabbed are 512 x 484 pixels by 256 gray scale. The wide variation between the aspect ratios will distort the cells to appear vertically elongated rather than round. This may be solved by duplicating each pixel twice in the x-axis, giving a viewing window of 320 x 350, or nearly a one to one aspect ratio. The gray scale intensities may shifted to the

right 4 bits, or divided by 16, and then mapped onto the 16 available colors. If desired, this pseudo-color mapping may also be used in a Video Graphics Adaptor (VGA) where the available 640 x 480 resolution no longer presents an aspect ratio problem, and the full raw image may be displayed.

In another embodiment, a "contour feature extraction" algorithm is used. There are two primary differences between the "reverse-fill" algorithm and the "contour feature extraction" approach. Both scan the image from top left to right for the first non-zero element. The contour algorithm then creates a vector map of the perimeter of the element by searching counter-clockwise for adjacent, unhighlighted pixels until the contouring is completed. The size of the element is then calculated from the vector map.

This technique, however, also highlights only the perimeter of each element, rather than every pixel in each element. This, combined with processing in the frame grabber 222 rather than disk swapping of raw image data, and the ability to perform a full-frame manually selected threshold binarization while executing the contour sizing/counting algorithm greatly improve throughput and eliminate errors due to element shape. The contour feature extraction algorithm works well with an even background, high contrast image. The algorithm, however, requires manual selection of threshold, and does not take into account any background light gradient or other filtering, all of which are desirable for automated scoring.

In another embodiment, the frame grabber 222 is used for real time image averaging. This technique sums a selected number of frames of image data on the fly, keeping the intermediate results in the second frame buffer. It has been found that, for images of interest in assay procedures, averaging two to four frames yields a substantial improvement in signal to noise (cells to background) allowing these images to be used without further filtering. This dramatically

increases throughput without adding processing overhead to the main computer.

It is also desirable in automated scoring to have the ability to perform localized auto-thresholding and binarization within windows of the image. This may be performed using software available from Coreco by performing statistical analysis on a window of user-specified size, for example 32 x 32 pixels, and deciding whether any cells exist in the window by looking at the peaks of the histogram of the window. If only one peak exists, than this is assumed to be the background peak, and no cells are in that region. If two peaks are detected, than a threshold is selected by a user-selectable percent distance between the background and foreground peaks, and this value is used to binarize the window. From here, the contour sizing/counting algorithm described above may be used to complete the autoscoring of the reaction wells 16.

In the most preferred embodiment, the DSP card 224 is used to perform high speed auto-thresholding and binarization. The DSP card 224 preferably includes a processor with a parallel high speed multiplier and adder and separate instruction and data busses.

As discussed briefly above, in one embodiment the frame grabber 222 captures the image and transfers them across the AT main computer bus in small blocks to a DSP input buffer. As the DSP receives the data, it performs all operations for determining the threshold automatically, binarizes the image, and compresses the elements to be sized and counted. As processing of each element is completed, the results are placed in the DSP output buffer. From there, the final sizing and counting are performed by the main computer.

More preferably, the data is transferred directly from the frame grabber 222 via a video bus. This frees the main computer for other tasks and takes full advantage of the multi-processor configuration.

AUTO-POSITION ALGORITHMS

Two different algorithms have been investigated. Both of the methods have been tested with positive results.

Method #1:

The objective of this method is to determine the location of the well within the image by locating the edge of the well within the image by locating the edge of the well and then its center.

Software obtains a profile or line of pixel values from each side of the image. In Figure 19, these lines correspond to the profiles of AB, BC, CD, and DA. The profiles for the image in Figure 19 are shown in Figure 20. In the BC profile, the left edge of the well is indicated by the black-to-white transition while the right edge is represented by the white-to-black transition.

When a profile is analyzed individually, it may be represented by one of four possibilities. A profile is defined as a Type 1 if all of its pixels have a "white" value. A profile is declared a Type 2 if all of its pixels contain two such transitions.

Type 1 - all white pixels
Type 2 - all black pixels
Type 3 - one transition (white-to-black OR black-to-white)
Type 4 - two transitions (white-to-black and black-to-white OR black-to-white and white-to-black)

After each profile is assigned a type, the types are analyzed collectively. The set of profile types determine the orientation of the object in the field of view. For example, Figure 21 shows the different orientations of the well if the profiles had a 1-3-2-3 ordering. Figure 22 shows the well orientations for a 4-2-2-2 ordering. The configurations of a

1-1-3-3 ordering is shown in Figure 23. Figure 24 shows the images corresponding to a 2-2-3-3 ordering of the profiles. Other orientations and, therefore, orderings are possible depending on the physical and geometrical relationships with the image and the object of interest, i.e., the bottom of the well.

After the orientation of the well is known, its coordinates in the image can be calculated. Since the physical characteristics of the well and the field of view are known a priori, the location of the transitions within each profile along with the well's orientation within the image provide sufficient information to determine the coordinates of the center of the well.

Method #2

This method bounds the image of the well to one dimension, finds the extreme of the well in that dimension, reposition the extreme to a known location by moving the tray, and repeats the process. The image of the well will be centered in the frame with only two iterations.

The software finds the extreme position of the well in the X-axis. (Point A on Figure 25). This is obtained by analyzing every horizontal profile in the image for an edge transition. Of all the observed edges, the software selects the edge with the smallest X coordinate. The software moves the well so the position of the edge is centered in the frame. Figure 26. The minimum or maximum position in the X-axis is determined again. Because of the geometric relationships of the well and the frame, this new position will represent the extreme position in the X-axis. (Point B in Figure 26). The well is move to locate the extreme position at a predetermined coordinate which will ensure that the well is centered in the frame.

AUTO-FOCUS ALGORITHM

The purpose of the auto-focusing function is to determine the maximum sharpness of the image. The basic operation is to capture an image, measure the sharpness, calculate the displacement in the Z-axis to the optimal focal point, and move the tray to the new position.

The following steps describe the auto-focus procedure:

1. Capture an image.

2. Create a region of interest (ROI) along the edge of the well. The ROI will constrain the area which the image processing is performed and reduce the time required for the computations. Figure 28 shows a region of interest superimposed on an image.

3. Execute a Sobel edge filter on the region of interest. This filter is a common image processing function which gives an estimate of the magnitude of the intensity gradient. Other filters such as the vertical and horizontal edges could be utilized.

4. Calculate a histogram on the region of interest. Determine a threshold for the histogram. To date, the value for the threshold is determined empirically. Additional research will lead to a dynamic threshold which is calculated for each image. Figure 29 shows a typical histogram and threshold for ROI.

5. Sum the number of pixels which have a value between the threshold and the maximum limit. The sum is a measure of the focus quality and will change as the degree of focus changes.

6. Once the focus quality measurement is known, the software compares it to previous measurements and calculates a displacement from the optical focal point. The tray is moved to this position and the steps are repeated until the focus quality is maximized.

CELL COUNTING ALGORITHM

The cell counting algorithm determines the number of cells present in an image.

The steps for the cell counting procedure is described below:

1. Capture an image.
2. Calculate the histogram of the image.
3. Calculate the threshold. Ideally, the value of the threshold would be set at location on the histogram as shown in Figure 30. To find the threshold:
   a. Average the histogram and calculate the first derivative.
   b. Average the first derivative and calculate the second and third derivatives.
   c. Searching from low to high intensity, find the intensity level where the values for the second and third order derivatives are between 0 and 1. This will ensure an area on the histogram void of abrupt changes.
   d. Starting at the intensity level found in Step C, and searching from high to low intensity, find the intensity level where the third order derivative changes from a negative value to a positive value. This value is the threshold and indicates the division of the background and foreground.
4. Scan the image for pixels with values equal to or greater than the threshold value. When a pixel is observed, contour the object with an edge tracking algorithm. Calculate the area for the object.
5. Compare the area of the object to the maximum and minimum limits for the area of a cell. If the object's area is within the range, classify it as a cell and remove it from the image.

6. Repeat steps 4 and 5 until all of the pixels in the image have been scanned.

7. Objects which exceed the maximum area limit may actually be multiple cells in close proximity and, therefore, must be considered. To address these objects, a de-clumping or decomposing algorithm has been developed. The cells have a positive intensity gradient extending from the perimeter to center, i.e., the center of the cell appears brighter than the edge. Figure 31 shows a typical grayscale contour of the two cells with a threshold value of less than 153. If the threshold is set to a value lower than the outmost region, the two cells would appear as a single object. If the threshold is increased, the cells would decrease in size and eventually appear as two separate objects. Figure 31 shows the two cells with a threshold set between 180 and 210.

To achieve the de-clumping effect, the current threshold is increased a given amount and steps 4-6 are performed. The cycle is repeated until certain conditions are met, for example, no objects are observed or the number of iteration is equal to set value such as five. Figure 32 shows the multiple thresholds calculated during this process.

AGGLUTINATION DETECTION DESCRIPTION

In one embodiment of the invention, the agglutination detection of the instrument uses a CCD camera to take an image of each well of a disposable similar to a standard 96 well microtitre plate. This image is digitized in such a manner as to result in at least a 8x8 pixel array by 8 level grey scale representation for each well, although 30x30 pixel array by 256 grey levels is the minimal recommended, and 512x484 by 256 resolution or greater may also be used. These images are shown in Fig. 33A through E, with 33A being a strong negative, 33B being a weak negative, 33C being a weak positive, 33D being a positive, and 33E being a strong positive reaction.

The digitized representation is then analyzed by taking at least 1 cross-section of intensity data (if only 1 section, this must be through the center), using low-pass filtering, if necessary, to eliminate noise, and a simple data replacement technique to remove rim information. This rim correction simply takes data known to be non-rim data by pre-calibrated position, but be close to a rim, and extend this value to its intensity transitions not caused by the result of chemical reactions. These processed intensities are shown in the top row of graphs in Fig. 34, with the X-axis being pixel positional information, and the Y-axis grey scale intensity.

Although it is possible to perform pattern recognition and image classification on intensity alone, this technique is subject to several weaknesses, including high positional repeatability requirements, intensity and geometric variations caused by different cell concentrations and pipetting inconsistencies, varying light sources, and time of reaction.

For these reasons, a derivative of the intensity is taken, and is the primary method of classification and scoring of these reactions. The derivatives are shown in the second row of graphs in Fig. 34. Note the monotonic descending values from strong negative to strong positives of these derivatives. By taking the sum of the absolute values of the peak negative-going and positive-going spikes of the derivative of each well, called the slope total, a numeric value associated with the relative "sharpness" of the center "button", any "halo" surrounding this button, and the backround of each well is generated independent of any absolute intensity values. This technique was developed as an expert system model of trained laboratory technicians' reading and scoring procedures.

The most important scoring task is discerning between a weak negative B and a weak positive C. The center intensity values are similar, and the button, halo and backround

transitions are more readily classifiable using the slope information from the derivative than using the absolute intensities.

Typical values for slope totals and center intensities are shown below. The range of both the Slope Totals and Center intensity values for each group is approximately plus/minus 10 units.

IMAGE DESCRIPTION	SLOPE TOTAL (0-100)	CENTER (0-255)	SCORE (0-4)
A Strong Negative	70	40	0
B Weak Negative	50	40	1
C Weak Positive	30	40	2
D Positive	20	55	3
E Strong Positive	10	70	4

As can be seen from these values, including the +/- 10 range, a scoring algorithm based on slope total information alone could distinguish, without overlap, between strong and weak negatives, and positives as follows:

Strong negative = Slope Total > 60
Weak negative = Slope Total < 60 AND Slope Total > 40
Positives = Slope Total < 40

To distinguish between weak positives, positives, and strong positives using slope totals alone, however, can lead to ambiguous scoring with a +/-10 range. If required, the center values allow for easier classification of positives:

Weak positive = Slope Total < 40 AND Center < 50
Positives = Slope Total < 30 AND Center > 50 AND Center < 62
Strong positive = Slope Total > 20 AND Center > 62

Actual cutoff values can be set by previous statistical stored information or on-board calibration wells, or both. Linear regression techniques can also be used for scoring using slope total and center values, and it is possible to adjust for run-to-run variations and reaction times with on-board controls. Neural nets can also be constructed using these parameters for scoring, as using the slope total as a primary discrimination tool allows for a robust and reliable detection environment not found in intensity-only based techniques.

OPERATION OF THE APPARATUS

Operation of the apparatus of the present invention is now described for HLA typing. The operator first isolates the cells of interests by known techniques (such as by the Ficoll Hypaque method). Since the reaction cartridges 10 will typically be provided with the reagents in a frozen state, the operator thaws the cartridge 10. The cartridge 10 preferably has a preprinted bar code containing assay type and other information. The operator then logs in the patient data by typing in the patient information using the microcomputer keyboard.

The operator places paramagnetic beads and a fluorophore into well 12 of cartridge 10 for dispensing by a pipette. 50 microliters of the sample cells are then pipetted manually by the operator into the sample well 11a, 11b or both. The operator then loads the cartridge 10 into the automated instrument in the load area 30. The pipette robot 34 retrieves and moves the cartridge 10 to a barcode reader to read the information on the preprinted cartridge barcode.

The pipette robot 34 transports the cartridge 10 and places it under a pipette. The pipette then adds 50 µl of paramagnetic beads and green fluorophore to the sample cells

from the well 12. A suitable green fluorophore is the 5,6 carboxyfluorescein disclosed in Table 1. The mixture is then incubated in the incubation area 38 for 10 minutes at the incubator's ambient temperature of 34°C +/- 2°C. The pipette robot 34 then retrieves the cartridge 10 and moves it to the pipette.

A magnet, such as a rare earth magnet (Permag, IL), is then placed approximate to the sample well to hold the cells which have now attached to the paramagnetic beads. The sample wells 11a, 11b are then washed to wash off uncaptured cells. 70 µl are aspirated from each of the sample wells 11a, 11b into the waste blotter and an equal volume of 70 µl buffer is added to sample wells 11a and 11b. This washing step is repeated 3 to 4 times, leaving a final volume of 100 µl.

The magnet is then removed and the cells are resuspended and mixed in the sample well 11a, 11b. 0.5 µl of cells are then pipetted into one of the reaction wells 16 on the cartridge 10. The cells are counted in this reaction well using a CCD and read at 490 nm. If the cell number is inadequate a signal is given to the operator and the cartridge is rejected. If the cell number is too high, the number of cells is estimated and must be diluted.

If the cell number was adequate or the cell number has been diluted, 0.5 µl of the cells are dispensed into each reaction well 16. The cartridge 10 is then moved to the incubator area 38, which is 34°C +/- 2°, for approximately 30 minutes. A rehydrated complement/red fluorophore mixture with 480 µl of buffer are provided to the pipette. A suitable red fluorophore is the propidium iodide disclosed in Table 1. The cartridge 10 is moved to the pipette which then dispenses 3 µl of the complement per reaction well 16. After all of the reaction wells 16 have been completed, the cartridge 10 is moved to the incubator area 38 and incubated for 30 to 45 minutes, depending on the samples being analyzed. Optionally, the pipette may dispense 50 µl of buffer per reaction well 16.

The cartridge 10 is then retrieved by the image robot 40 and each well is image processed at 490 nm/540 nm and the results and/or image is then stored. After the samples have been image processed (the cells have been counted and scored), the cartridge 10 is then moved to an unload area 46 where it is unloaded manually by an operator.

Examples

The following examples are given to illustrate more specifically use of the apparatus and methods of the present invention.

Example 1 HLA Typing by Two Color Fluorescence Using Complement Dependent Microlymphocytotoxocity For Image Analysis

Referring to Figure 1, wells 11a and 11b designate reservoirs for holding leukocyte suspension for which a HLA determination was to be carried out. Lymphocyte purification was carried out using paramagnetic particles purchased from Advanced Magnetics Inc. (Cambridge, MA) (under the BIOMEG tradename) conjugated with CD2, or CD8 monoclonal antibodies according to the published procedures (Vartdal F. et al., Tissue Antigen 1986; 28: 30-1312). For Class II typing, monoclonal antibody such as L243 could be conjugated to similar paramagnetic particles purchased from Advanced Magnetics Inc. (Cambridge, MA). After the initial manual loading of purified lymphocyte suspension into one of the two sample wells 11a or 11b, all subsequent steps were handled by the apparatus of this invention. Reagents including typing sera, paramagnetic particles and 5,6 carboxyfluorescein diacetate (Sigma, MO) mixture, lyophiled complement (Pel Freeze, Milwaukee, WI) and propidium iodide (Sigma, MO) mixture that were necessary to complete a Class I or II HLA Typing were included on the cartridge 10. A volume of 100 µl of the paramagnetic particles and 5,6 carboxyfluorescein

diacetate mixture was pipetted into 100 µl of lymphocyte sample. After 10 minutes incubation at room temperature, the stained and rosetted cells were then separated from the uncaptured leukocytes by placing the underside of the well against a rare earth (Permag, IL) magnet for 15 second. Rosetted cells were subsequently washed with three changes of 300 µl of 1 TDX® buffer (Abbott Labs, IL) while keeping the rosettes in place by the above-mentioned magnetic device. A minimum of 0.5 µl of rosetted leukocytes was pipetted into reaction wells 16 containing at least 0.5 µl of HLA typing serum submerged in 2.5 µl of mineral oil. At the end of the 30 minute incubation period, a minimum of 3 µl of rabbit serum (complement) containing 2 mg/ml of Propidium iodide was added to each of the reaction wells 16. The reaction was allowed to incubate for an additional 30 minutes at room temperature. Positive reactions were indicated by varying degree of lympholysis. 5,6 CDF stained cells were viewed under a set of excitation (450 to 490 nm) and emission (520 to 560 nm) filter (Nikon, Japan), while the PI stained cells could be observed using a set of excitation (510 to 560 nm) and emission filter (590 nm).

Example 2 Immunocytochemical Staining of Labeled Cells for Biologic Markers in Biopsy Materials Or Tissue Sections

In another assay, human estrogen receptor expression on normal and malignant breast tissues using immunoperoxidase cytochemical method was used. Tissues were harvested and prepared according to the Abbott-ER- ICA Monoclonal Assay (Abbott Labs, Abbott park, IL) using immunoperoxidase reaction. The nuclei of the cells that did not contain a significant amount of estrogen receptor would show up light blue. In contrast, tumor cells with elevated estrogen receptor expression would appear reddish brown. Applications of this technique can be extended to other cellular, or

subcellular biologic markers in conjunction with insitu hybridization technique using DNA/RNA probes or other immunostaining methodologies including various isotopes, chemical stains, immunologic reagents, or enzyme/substrate combinations. Biologic markers can include protein, carbohydrate, lipid or any of these combinations. Specimen can either be a blood smear; biopsy materials or cytologic smears; or thin tissue sections prepared by chemical fixation, frozen, or paraffin section methodologies according to standard methodologies.

Example 3 Front Surface Immunoassay For Analyte Determination

As discussed in more detail below, a significant advantage of the present invention is the ability to upgrade the device to perform different types of assays. For example, the apparatus and method of the present invention may be used to enhance the precision and sensitivity of fluorometric or coloramatric immunoassays. In one example of a different assay type, reactions are carried out in a 96 well microliter-carriage (Abbot Labs, Abbott Parks, IL.) Reagent mixing, incubation and signal development occur in the reaction wells. In the sample reaction, paramagnetic particles are coated with mouse IgG by procedures known to those skilled in the art. Goat anti-mouse labeled with B-Galactosidase is used for detecting the mouse IgG. To start a reaction, 50 µl of mouse IgG coated paramagnetic particles are mixed with equal volume of goat anti-mouse-B Galactosidase complex in the reaction wells for twenty minutes at room temperature. The unbound goat anti-mouse-B Galactosidase complexes were washed away with a total of 500 µl of TDX® buffer (Abbott Labs, Abbott Park) while the paramagnetic particles are held in place with a magnet. A volume of 50 µl of a fluorogenic substrate such as Di-B-Galactosylfluoroscein (Sigma, MO) are added to the particles. Fluorescence densitometry or absorbance changes

may be monitored through the image analysis arrangements described above.

Example 4 Detection of Hepatitis B Surface Antigen Through Agglutination

Agglutination assays were performed in accordance with the instructions provided using the Abbott Auscell® kit commercially available from Abbott Laboratories, North Chicago, Illinois 60064, which contains reagents and 96-reaction well v-bottom agglutination plates. The Abbott Auscell® kit instructions are hereby incorporated by reference and provide for reversed passive hemagglutination for the detection of Hepatitis B surface antigen. Lyophilized antibody-sensitized duracyte cells are reconstituted with reconstitution solution. 25 µl of specimen dilution buffer were then added to each reaction well. 2 µl of test serum were added to appropriate wells. 25 µl of the antibody-sensitized duracytes were then added to each reaction well. The reactants in the reaction wells were mixed by tapping the sides of the plate or carriage tray, the plates were incubated without vibration for two hours and then read using instrumentation used in the present invention.

FIELD UPGRADES

The apparatus and method of the present invention provide significant advantages over the prior art devices. These advantages have been described in part throughout the text of the above description. Another significant advantage, which is described in more detail here, resides in the expandability of the apparatus to perform different assays, and the minimum amount of modifications which must be made to upgrade the apparatus in order for the device to perform different assays.

As discussed above, variation in sample preparations

and other anomalies limit the usefulness of most, if not all, available systems since these systems require major hardware redesign to accommodate these variations. Further, available automated assay instruments are dedicated to a single type of assay. Again, major hardware redesign is needed to upgrade the instrument to perform assays other than the one it was originally designed for.

The system of the present invention provides an arrangement which does not have these limitations. The apparatus and method of the present invention can be easily reconfigured to accommodate variations in an assay test or to perform different assays. The modification will simply require changing the optical filters and objectives and/or modifying the algorithms for the image processing and other minor modifications. The algorithm can be developed and the appropriate filters and objectives selected before the field upgrade is performed. As will be appreciated these modifications or upgrades can then be performed in the field without a significant amount of effort by the person performing the upgrade in the field.

Since the assay steps are performed automatically, a significant amount of human operator time is also eliminated. It is expected that an HLA assay performed using the instrument of the present invention will result in a saving of between 63%-80% of the operator time required to perform the steps manually.

The reader on the instrument could be adopted to read fluorescence, agglutination, absorbance and chemiluminescence assays. Also, cell morphology could be determined. Other assays could require higher resolution and better sensitivity and stability. This could be overcome with different cameras which require different computer hardware and even more processing time.

The foregoing description of the preferred embodiments has been presented for purposes of illustration

and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed. It is intended that the scope of the invention be defined by the following claims including all equivalents.

CLAIMS

1. A liquid dispensing mechanism comprising:

a dispensing probe having a tip for dispensing the liquid;

a pump for selectively discharging fluid from the probe tip, the pump operable to dispense fluid in droplets from the probe tip;

means for detecting when a droplet of the liquid has formed on the tip of the probe; and

means for detecting when the droplet formed on the probe tip has separated from the probe tip.

2. The liquid dispensing mechanism according to Claim 1 wherein the mechanism has means for determining when the dispensing probe has been inserted into either of two liquids having closely related dielectric constants.

3. The liquid dispensing mechanism according to Claim 2 wherein the two liquids are oil and serum.

4. The liquid dispensing mechanism according to Claim 1 wherein means are provided for partial droplet formation on the dispensing probe tip in air before the dispensing probe tip is inserted into a receiving liquid.

5. A liquid dispensing mechanism comprising:

a dispensing probe having a tip for dispensing liquid;

a pump for selectively discharging measured units of fluid under sufficient force from the probe tip to inject the droplet into a receiving liquid having a liquid/liquid interface to a predetermined depth within the liquid;

means for determining when the dispensing probe tip has reached a preprogrammed insertion level of the receiving liquid before injection of the droplet into the receiving liquid; and

means for detecting when the droplet has been injected into the receiving liquid.

6. The liquid dispensing mechanism according to Claim 5 wherein positioning of the dispensing probe tip in relationship to the interface is predetermined before injection of the droplet.

7. A method for dispensing liquid from a dispensing probe tip into a receiving liquid in a container, comprising:

positioning the probe tip at a known or predetermined position in or near the receiving liquid;

forming the dispense fluid into a droplet on the probe tip in the receiving liquid;

detecting when the droplet of the liquid is formed on the tip of the probe; and

detecting when the droplet formed on the probe tip has separated from the probe tip.

8. The method according to Claim 7 wherein separating of the droplet from the probe tip is achieved by forming the droplet on the probe tip after the dispensing probe tip has been inserted into the receiving liquid and separating the droplet from the probe tip by the action of the probe tip being pulled from the receiving liquid.

9. The method according to Claim 8 wherein the receiving liquid is comprised of oil and serum having an interface therebetween and wherein the droplet is formed such that contact of the droplet with the interface between the oil and serum is achieved; upon withdrawal of the dispensing probe

tip, the formed droplet which is in contact with the oil serum interface adheres sufficiently to the interface to be pulled from the dispensing probe tip upon withdrawal.

10. A method for injecting a dispensing liquid from a dispensing probe tip into a receiving liquid in a container, comprising:

positioning the dispensing probe tip at a predetermined depth within the receiving liquid;

forming and injecting a measured droplet of liquid from the dispensing probe tip into the receiving liquid under a programmed force to achieve transfer of the liquid droplet to a predetermined depth within the receiving fluid; and

withdrawing the dispensing probe tip from the receiving liquid.

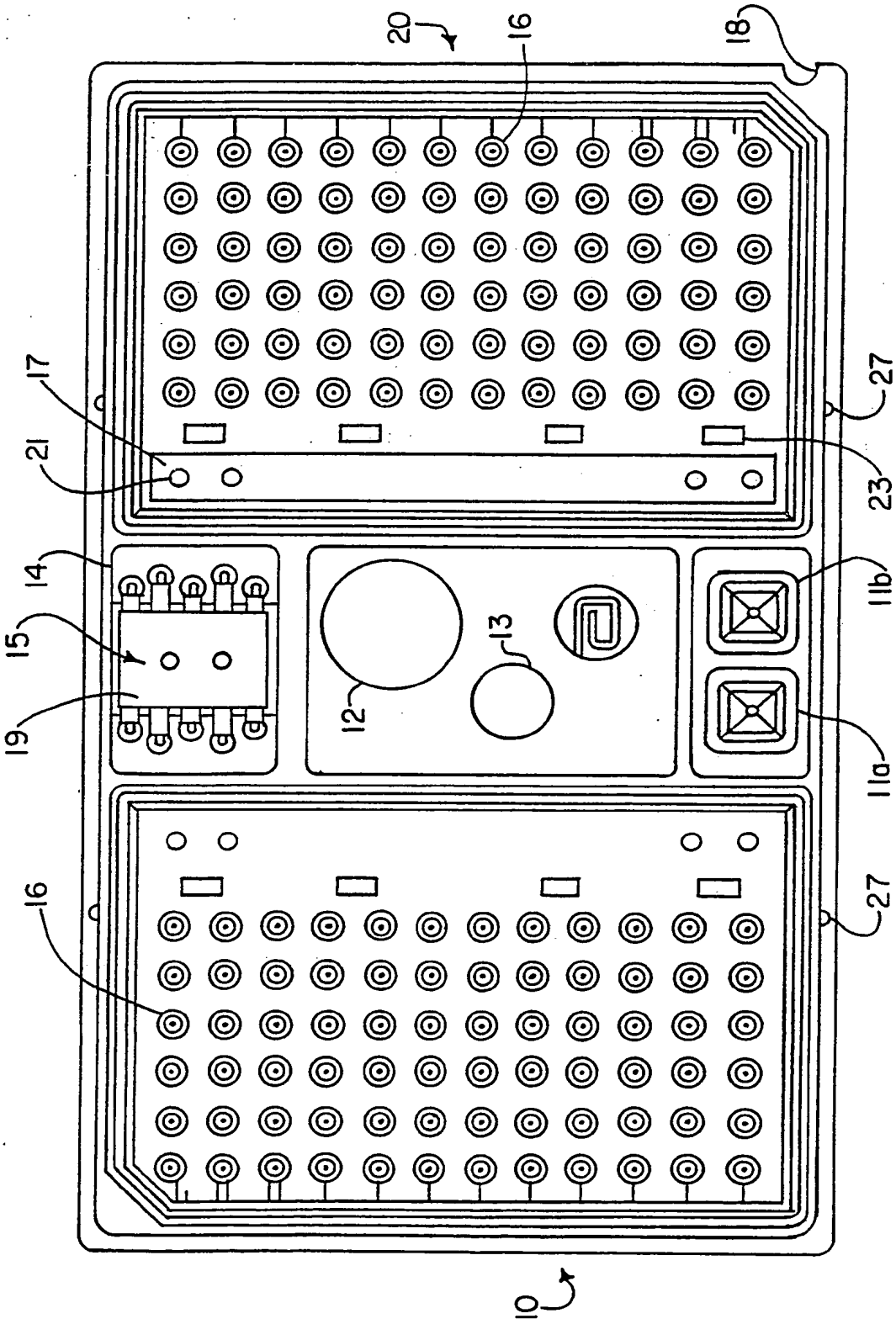


FIG. 1

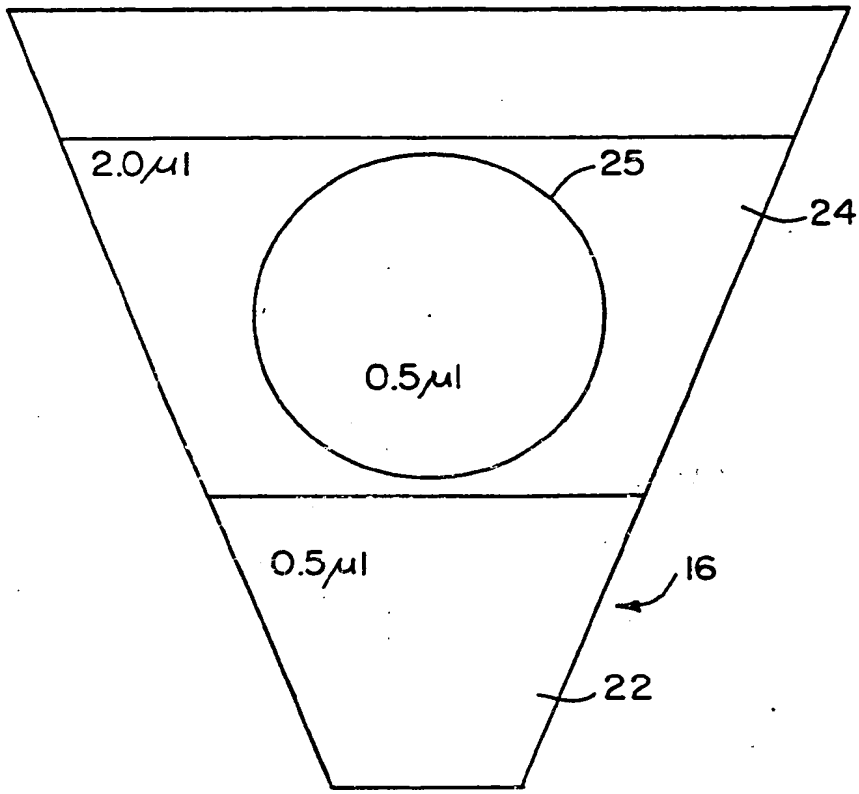


FIG. 2

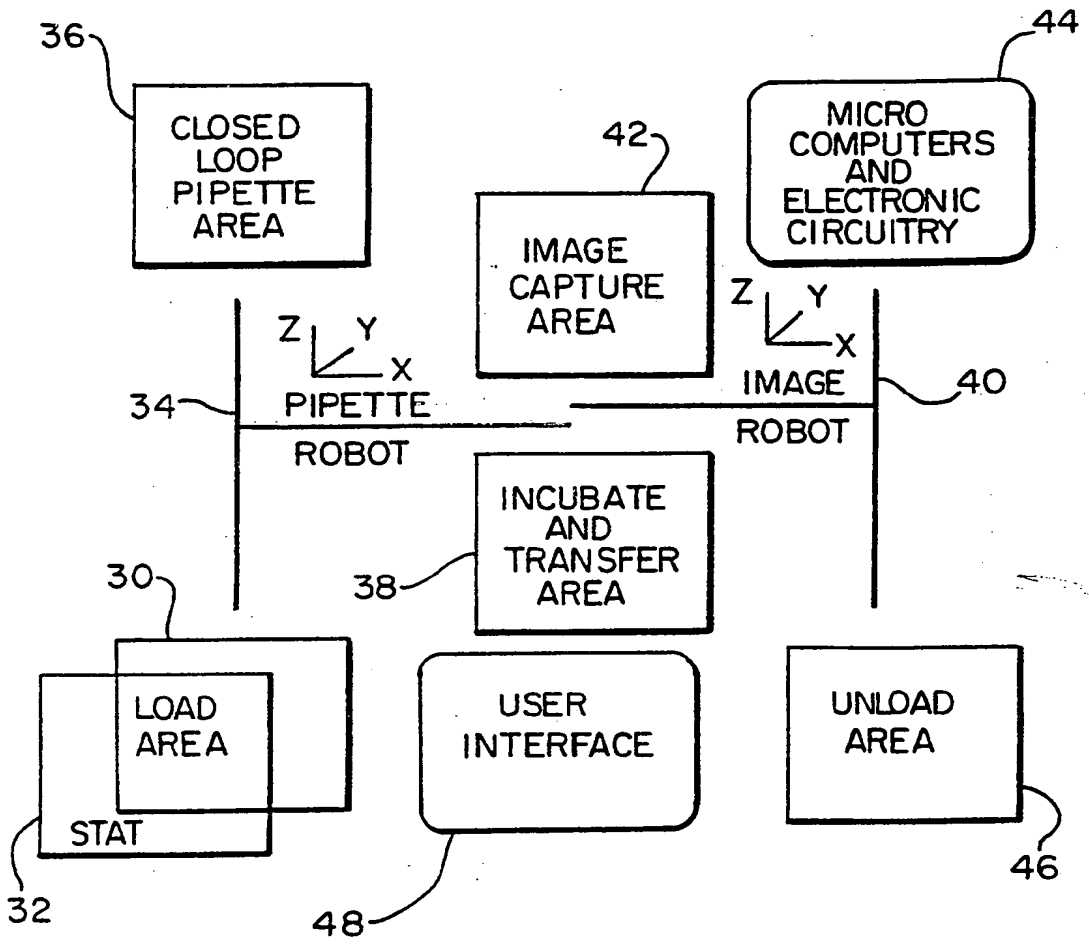
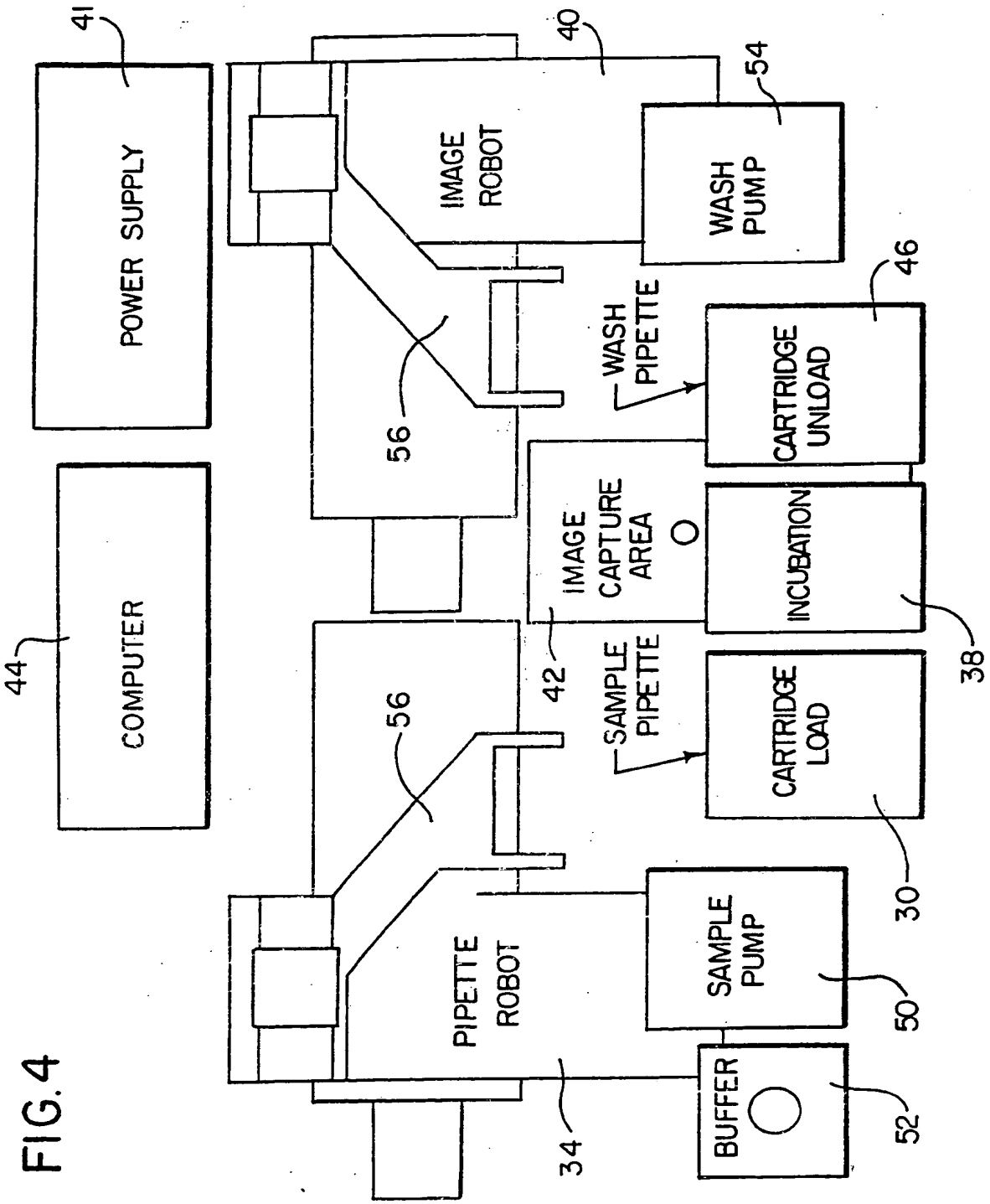

36
CLOSED LOOP PIPETTE AREA
42
IMAGE CAPTURE AREA
44
MICRO COMPUTERS AND ELECTRONIC CIRCUITRY
Z
Y
X
34
PIPETTE ROBOT
IMAGE ROBOT
40
INCUBATE AND TRANSFER AREA
38
30
LOAD AREA
STAT
32
USER INTERFACE
48
UNLOAD AREA
46

FIG. 3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POWER SUPPLY
40
IMAGE ROBOT
54
WASH PUMP
46
WASH PIPETTE
CARTRIDGE UNLOAD
56
IMAGE CAPTURE AREA
INCUBATION
38
44
COMPUTER
42
SAMPLE PIPETTE
CARTRIDGE LOAD
56
30
PIPETTE ROBOT
SAMPLE PUMP
50
BUFFER
52
34

FIG.4

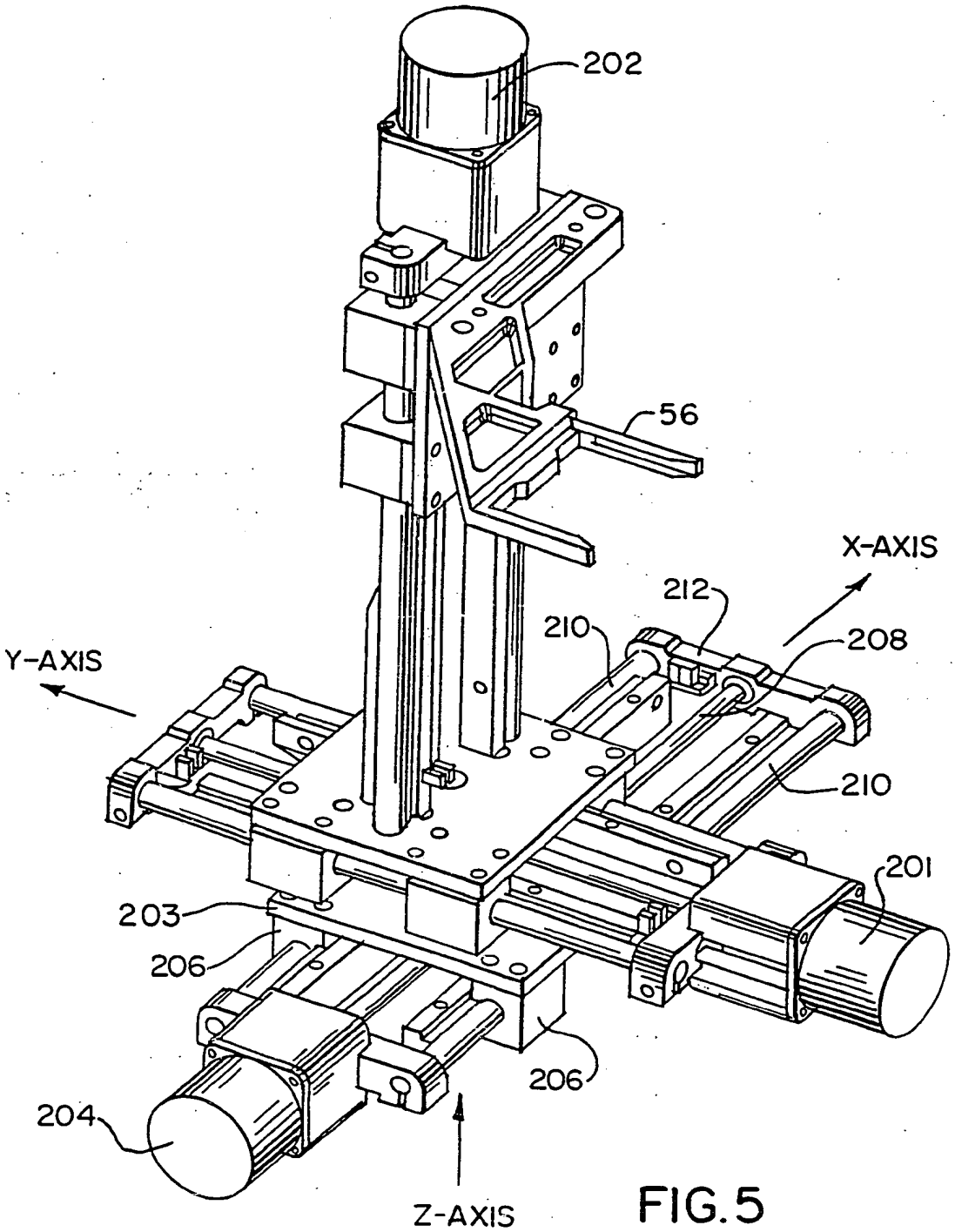

FIG.5

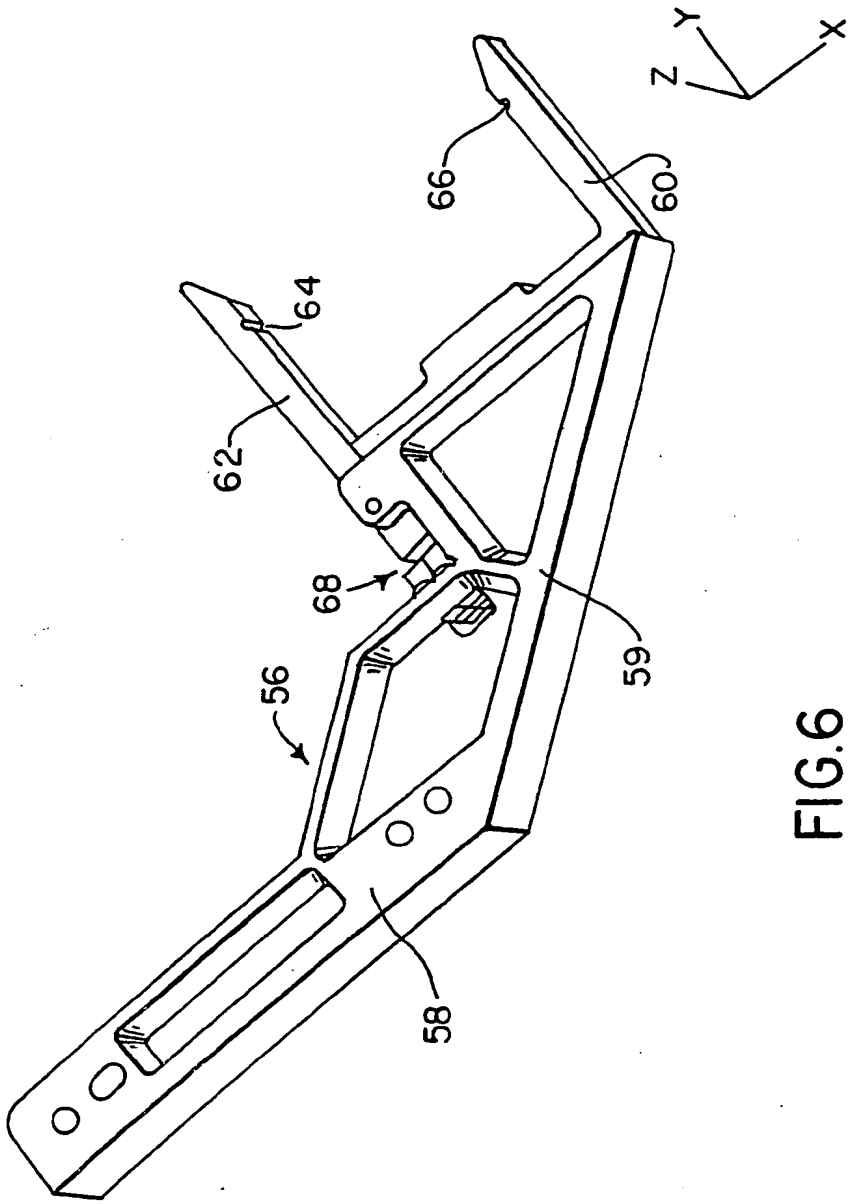

FIG.6

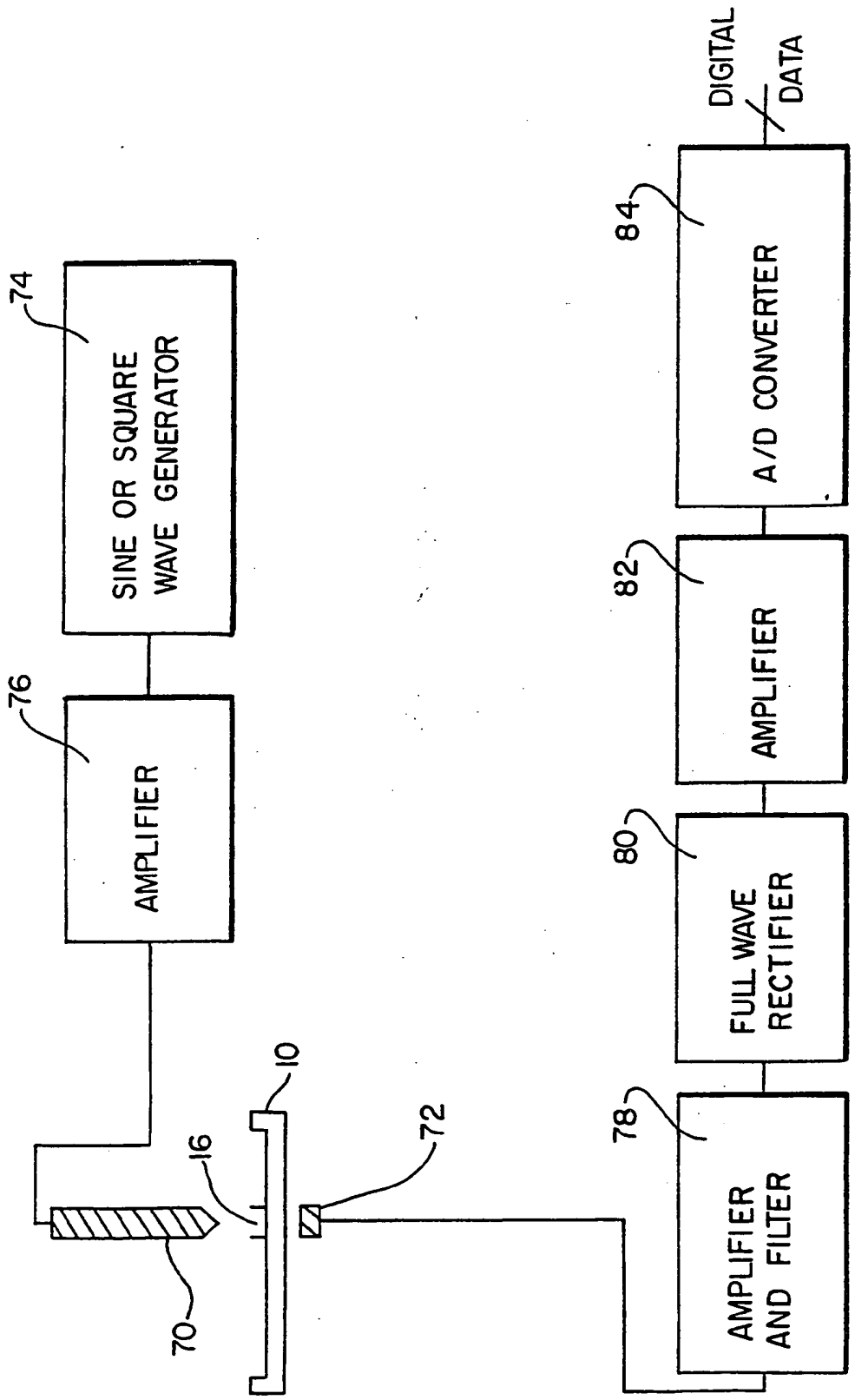
SINE OR SQUARE WAVE GENERATOR
74
AMPLIFIER
76
10
16
70
72
AMPLIFIER AND FILTER
78
FULL WAVE RECTIFIER
80
AMPLIFIER
82
A/D CONVERTER
84
DIGITAL DATA

FIG. 7

FIG.8

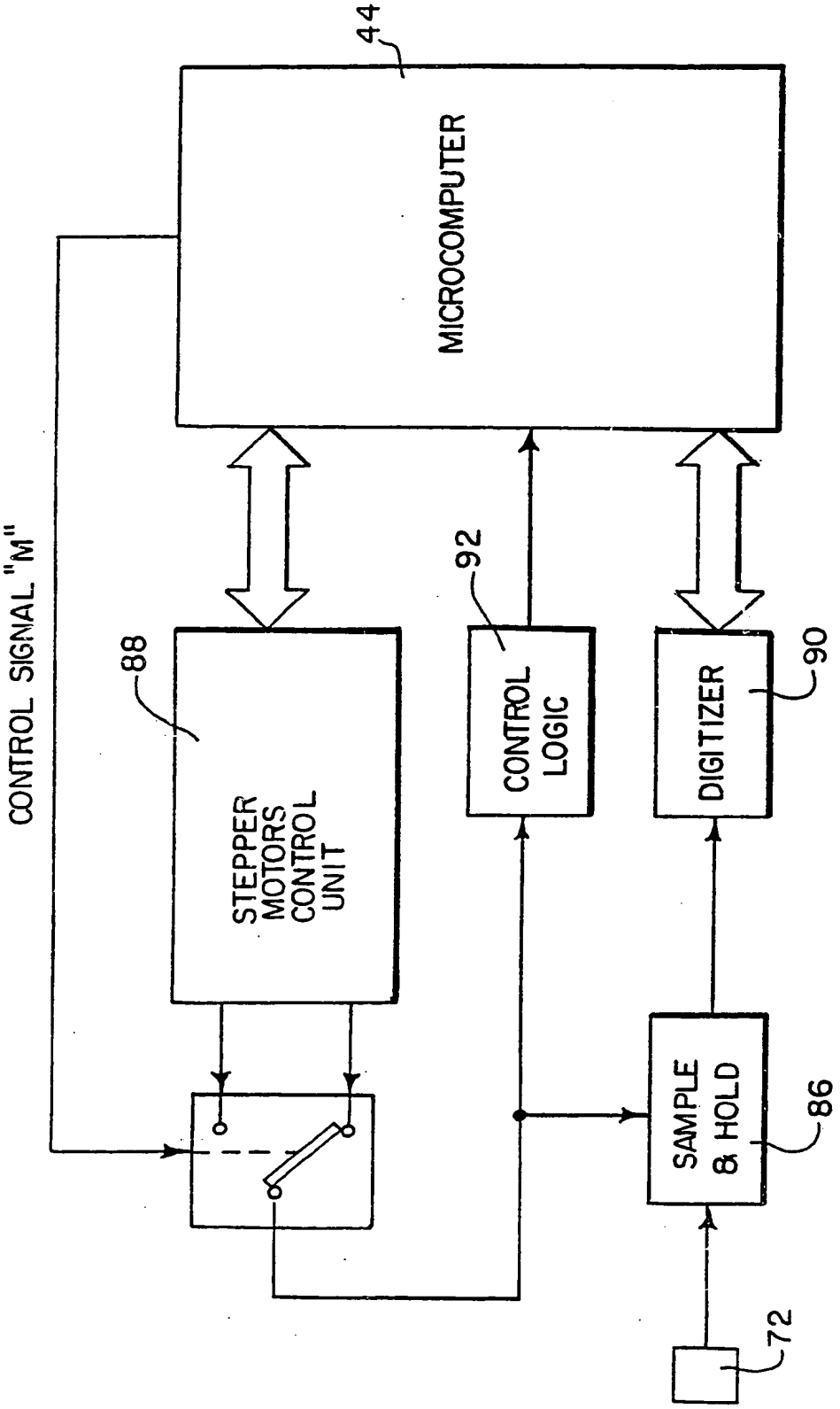
44
MICROCOMPUTER
CONTROL SIGNAL "M"
88
STEPPER MOTORS CONTROL UNIT
92
CONTROL LOGIC
90
DIGITIZER
86
SAMPLE & HOLD
72

FIG.9

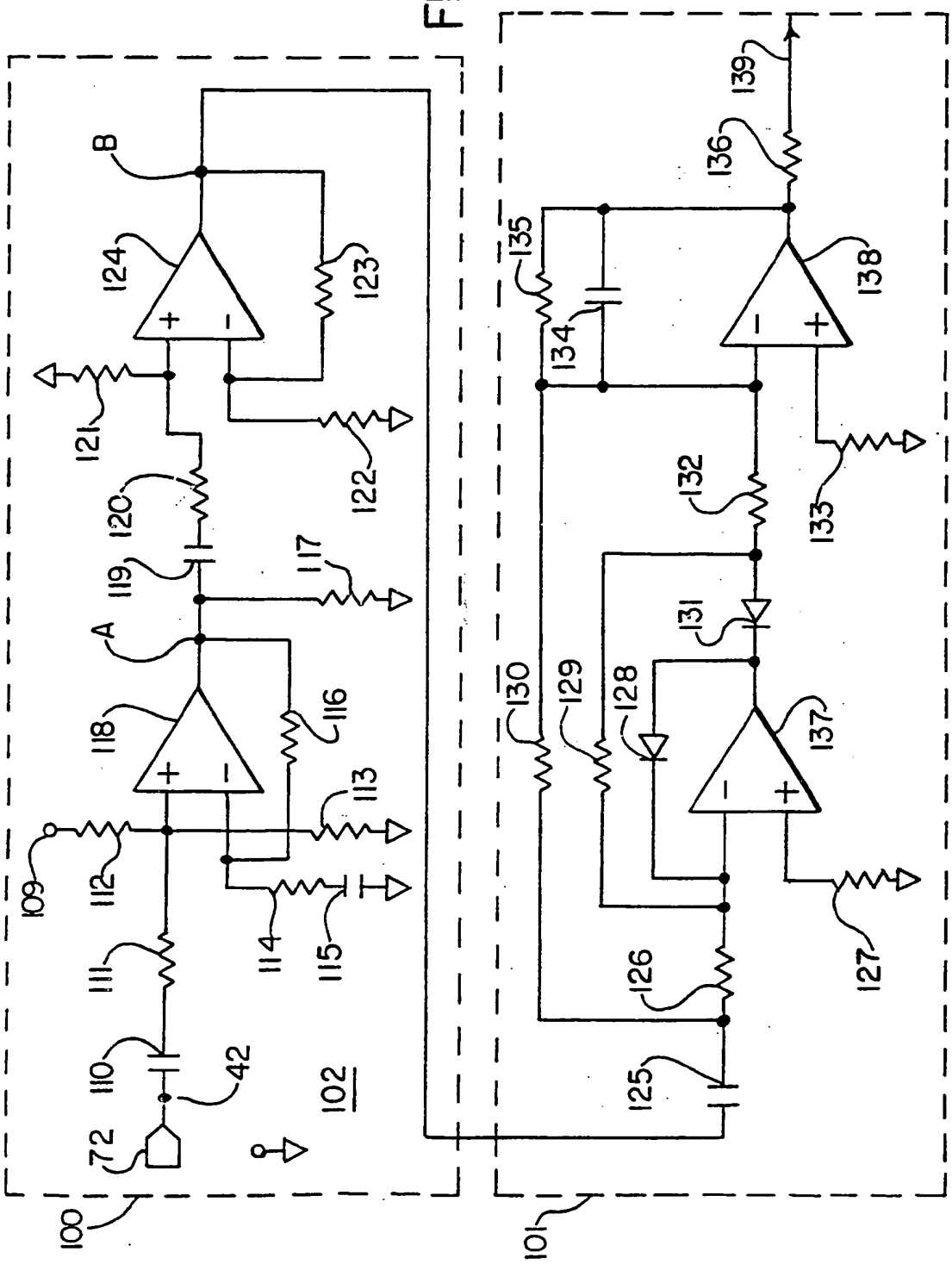
100
101
102
A
B
42
72
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FIG. 10

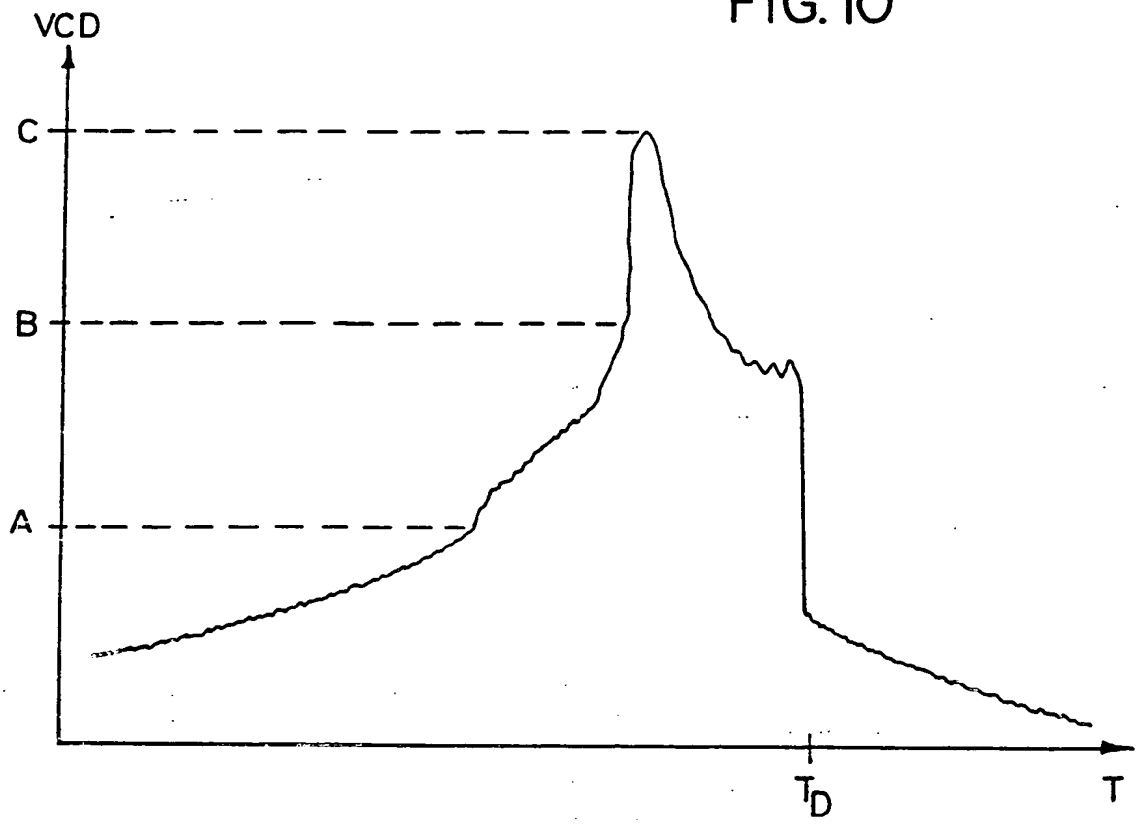
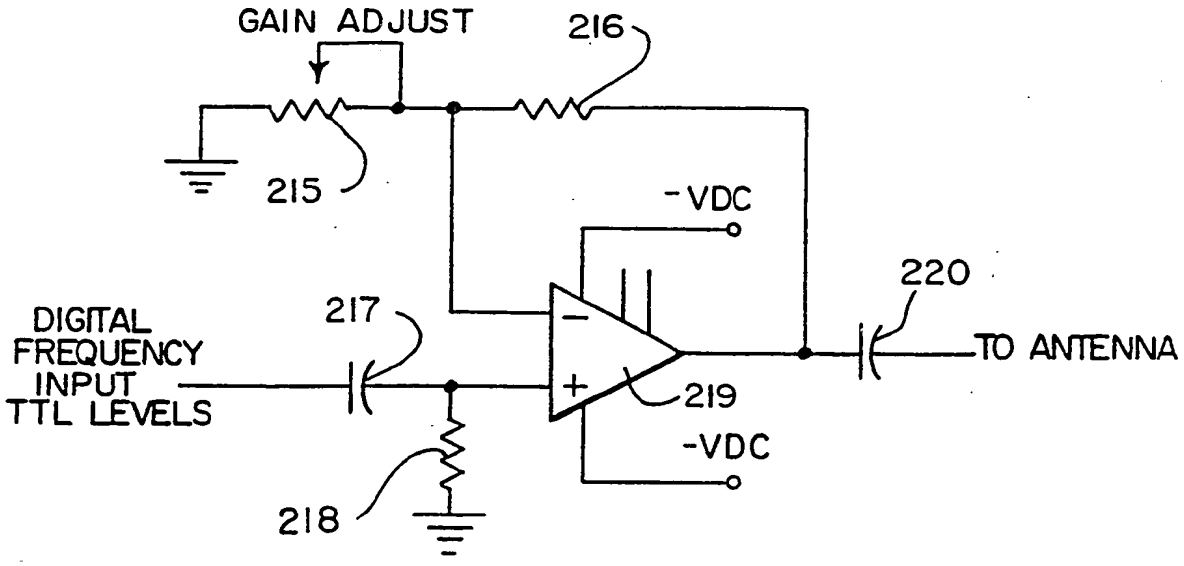

VCD
C
B
A
T_D
T

FIG. 11


GAIN ADJUST
216
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-VDC
220
DIGITAL
FREQUENCY
INPUT
TTL LEVELS
217
219
TO ANTENNA
-VDC
218

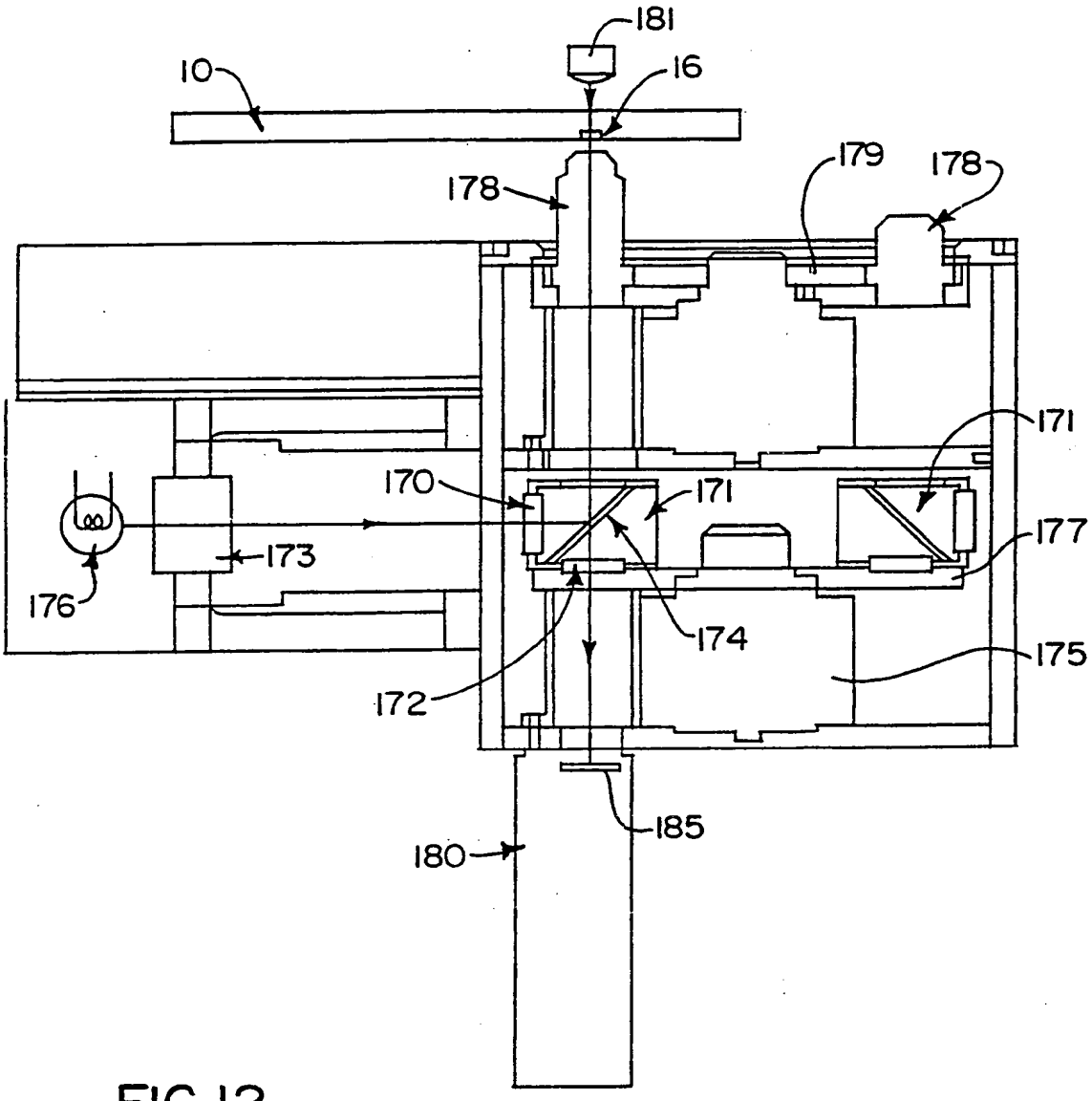


FIG.12

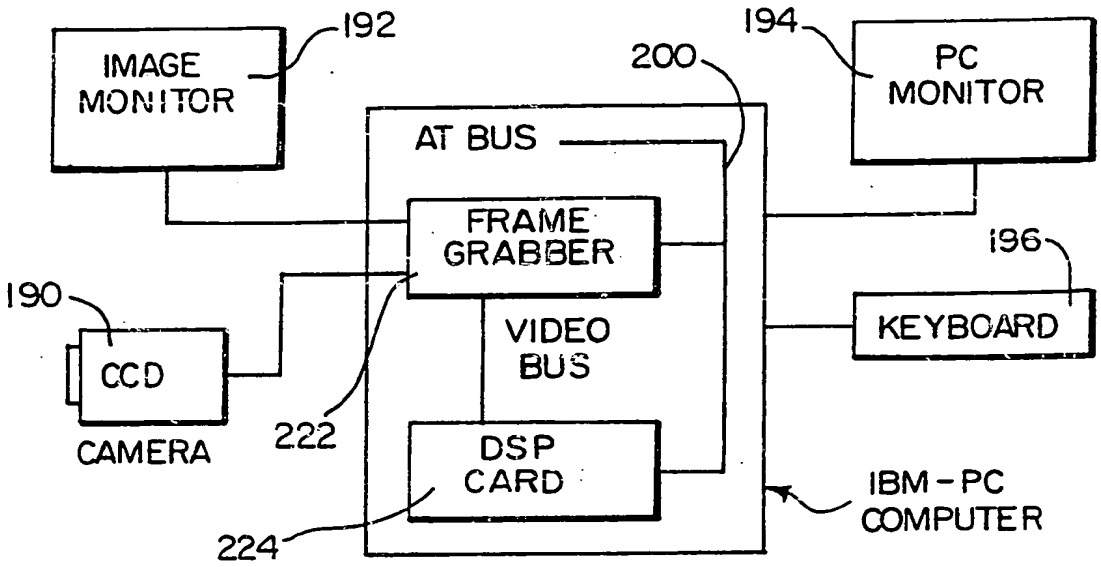
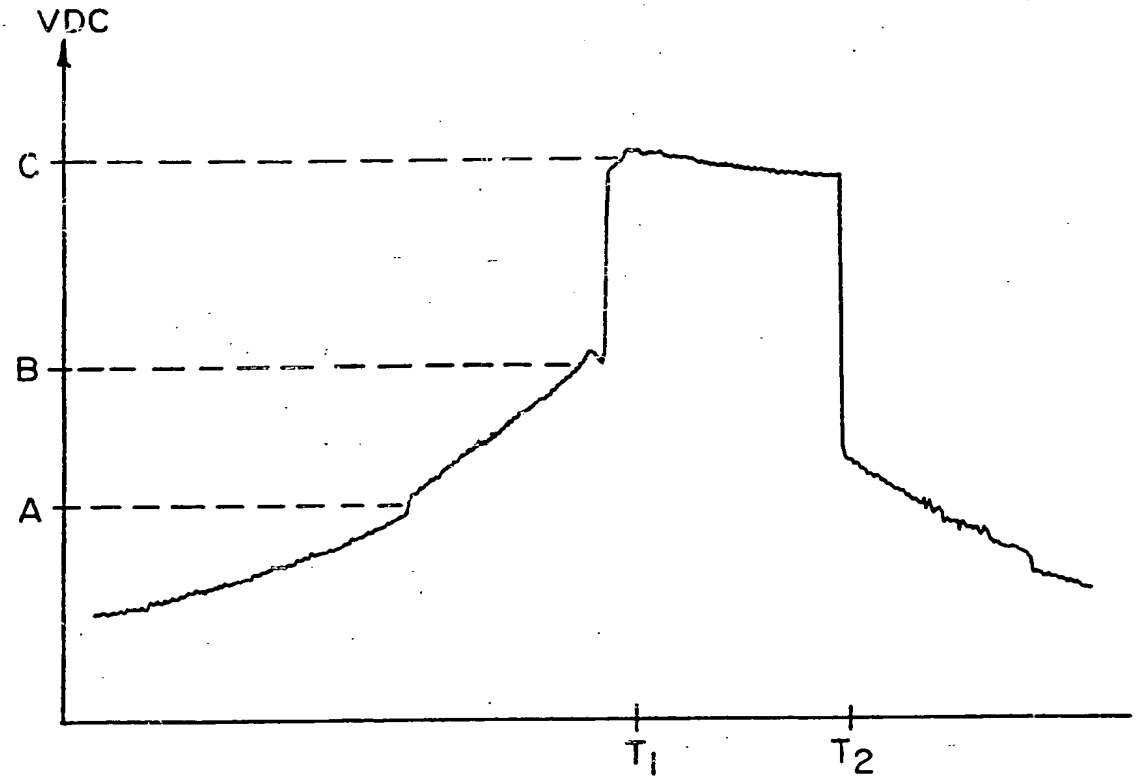


IMAGE MONITOR
192
194
PC MONITOR
200
AT BUS
FRAME GRABBER
196
190
KEYBOARD
VIDEO BUS
CCD
CAMERA
222
DSP CARD
IBM-PC COMPUTER
224

FIG. 13


VDC
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T1
T2

FIG. 14

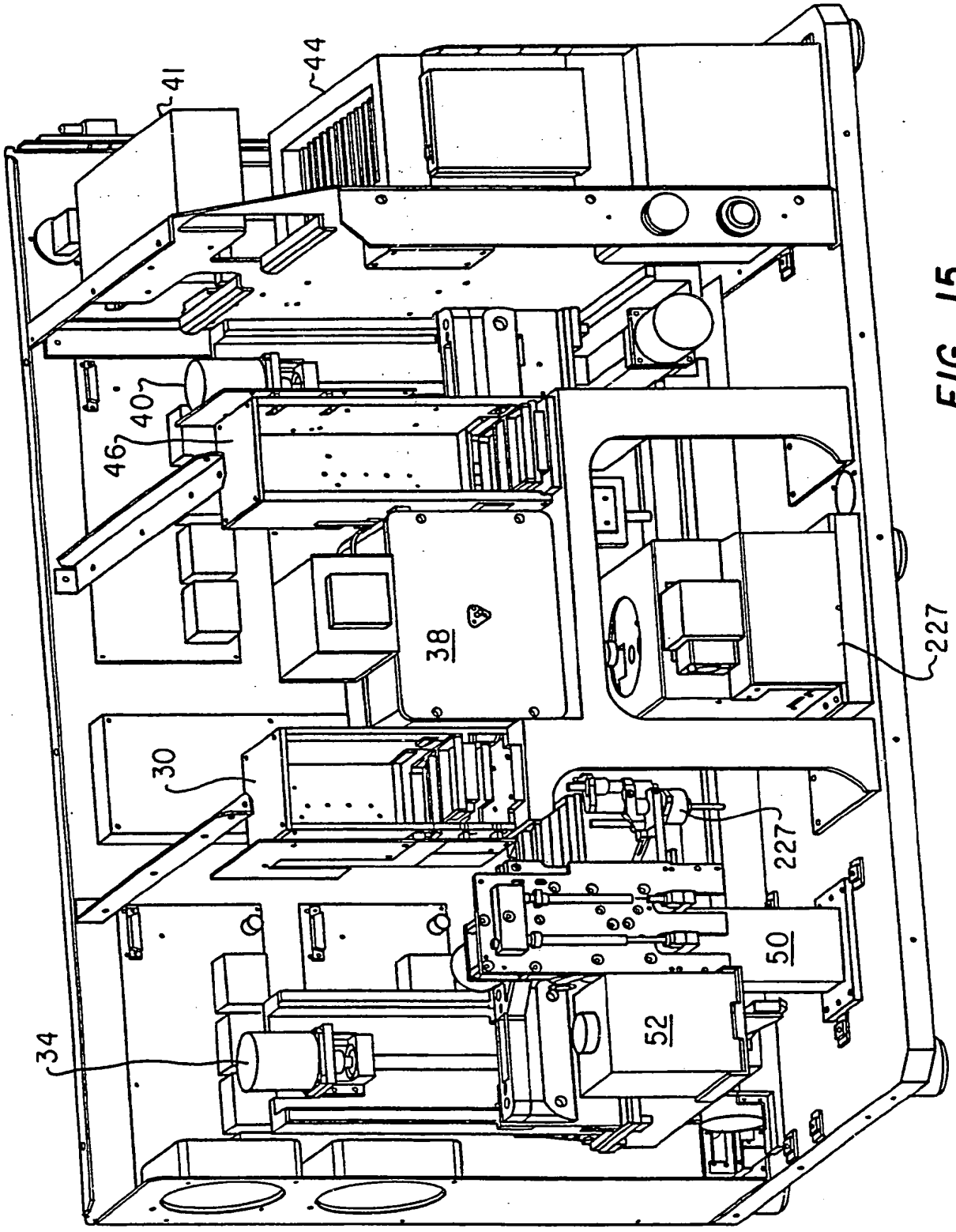


FIG. 15

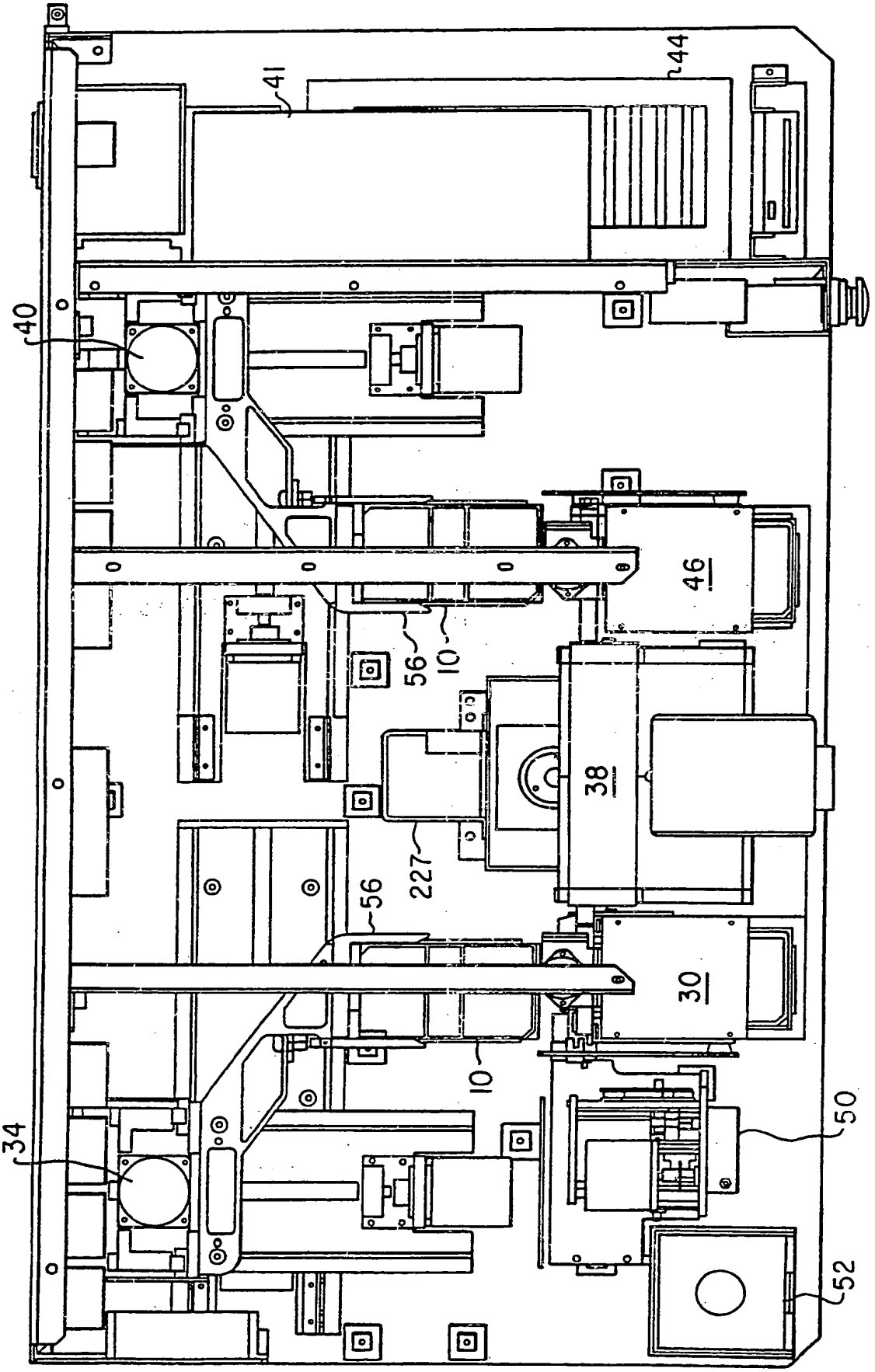


FIG. 16

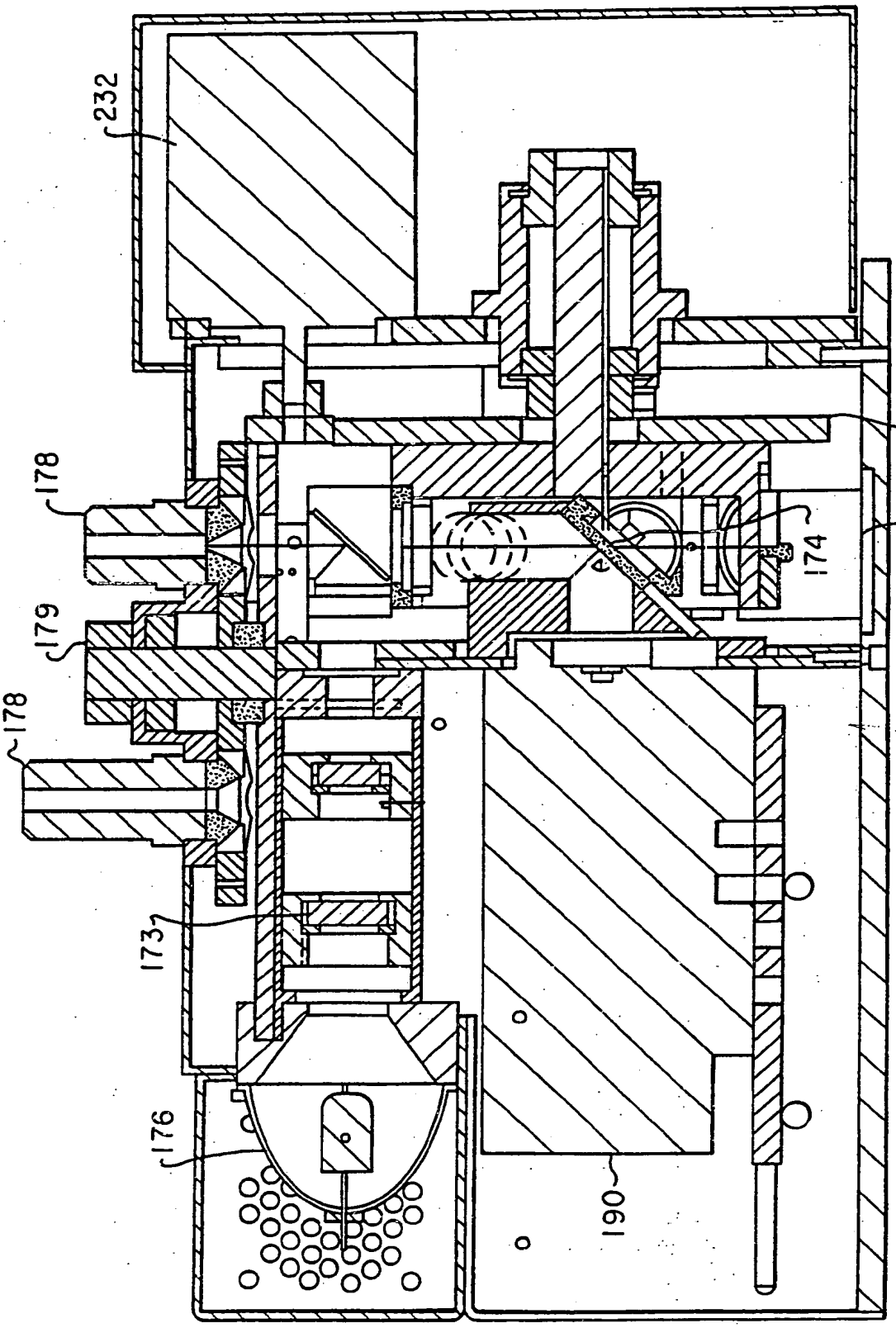
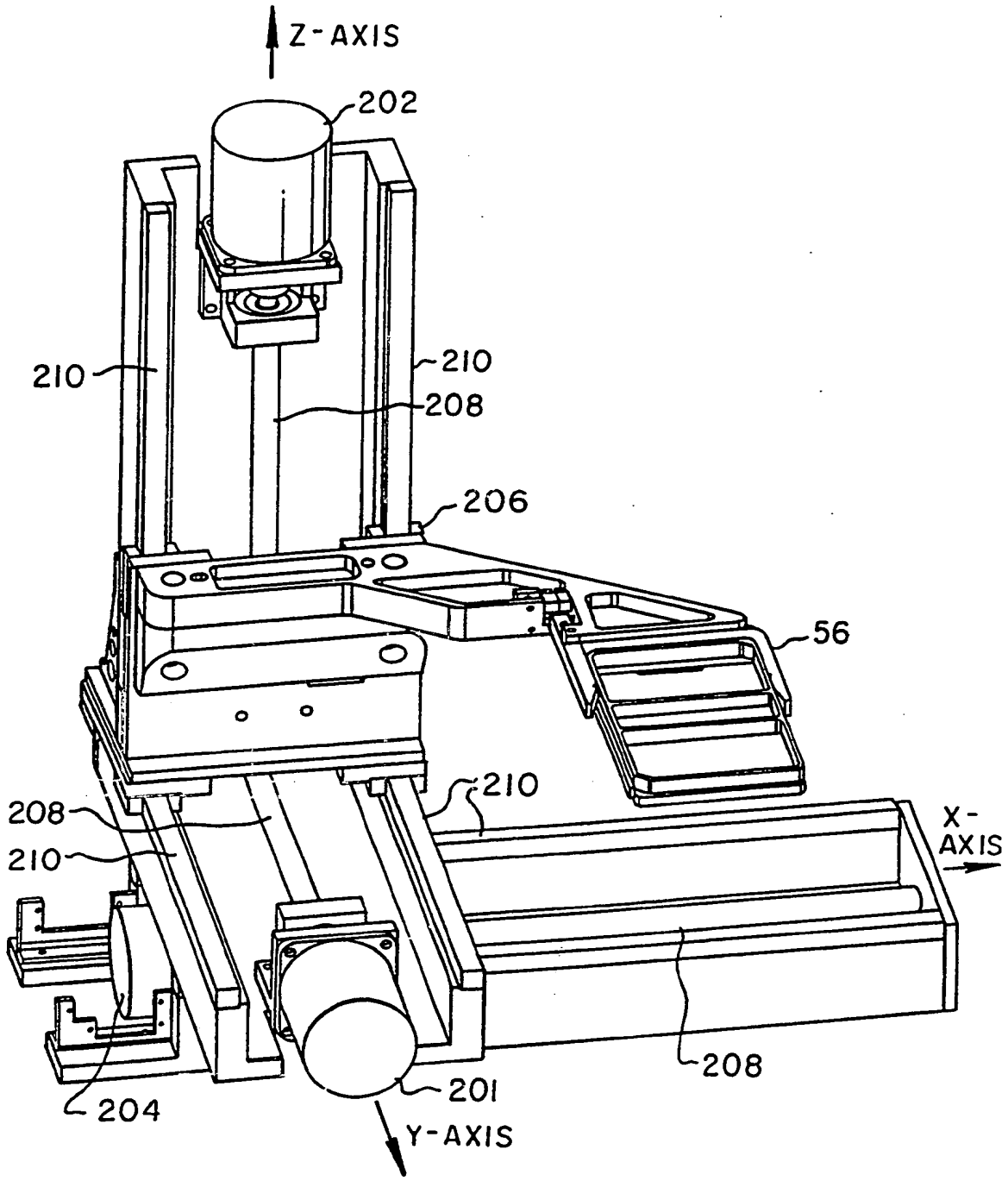


FIG. 17


Z-AXIS
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X-
AXIS
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201
204
Y-AXIS

FIG. 18

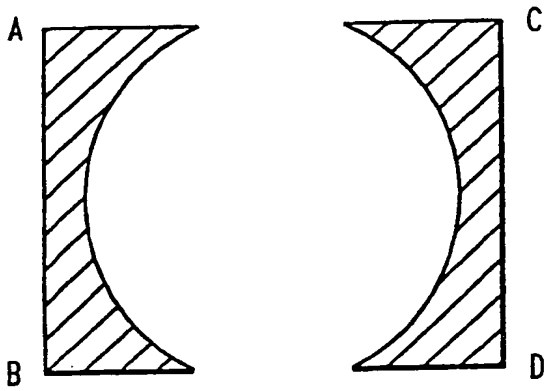
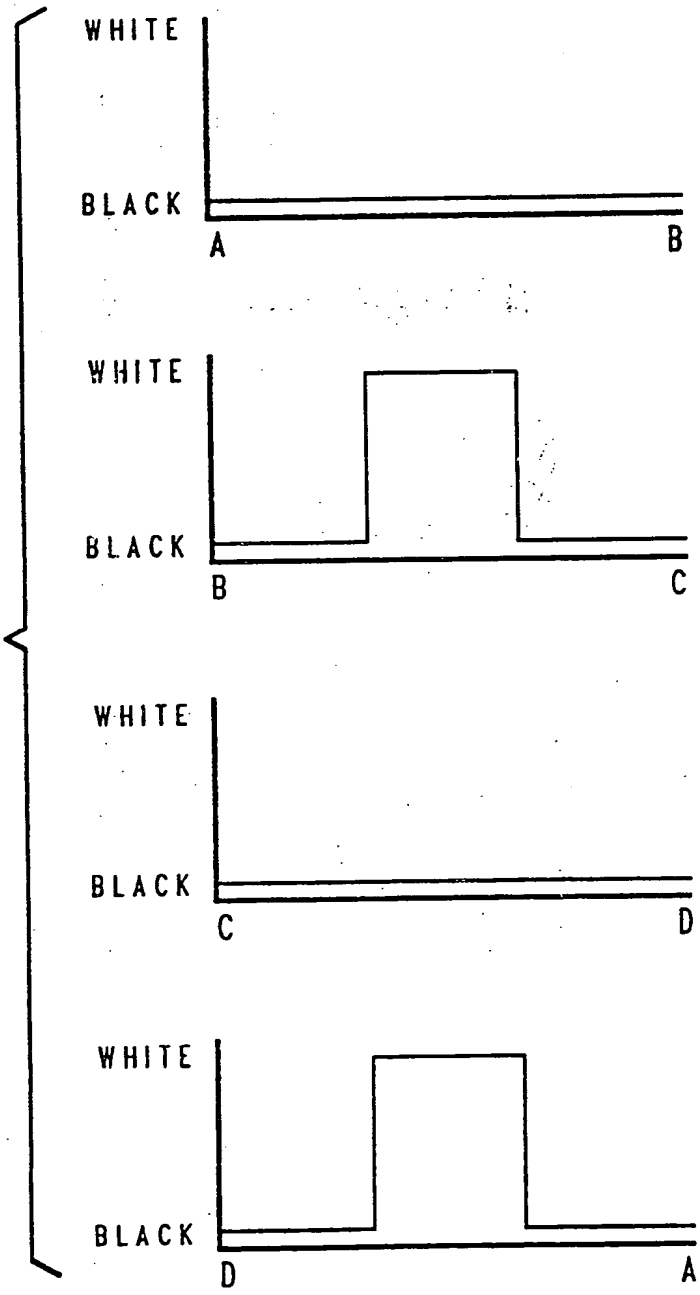

A
B
C
D

Fig. 19


WHITE
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WHITE
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C
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D
WHITE
BLACK
D
A

Fig. 20

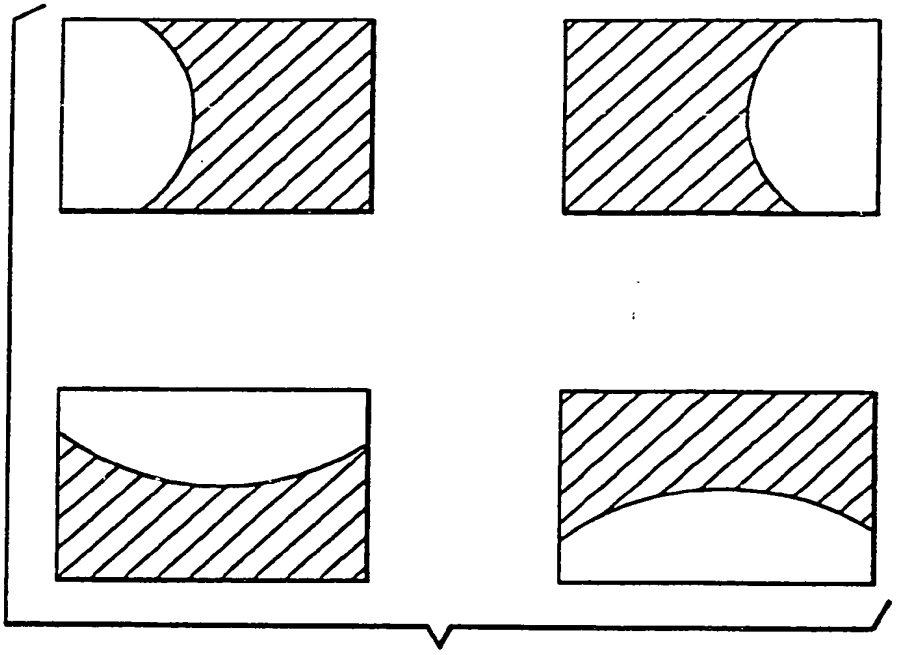

Fig. 21

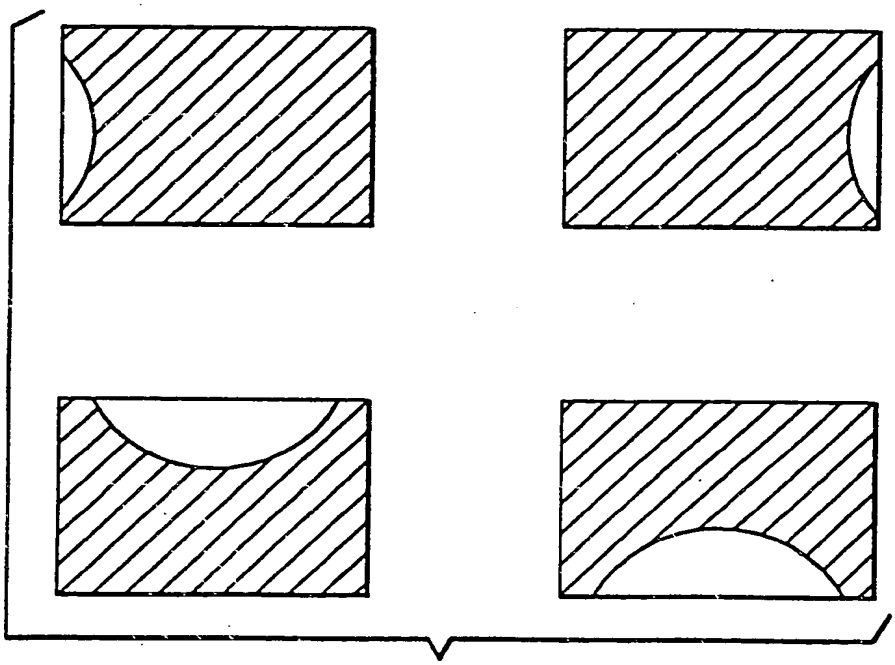

Fig. 22

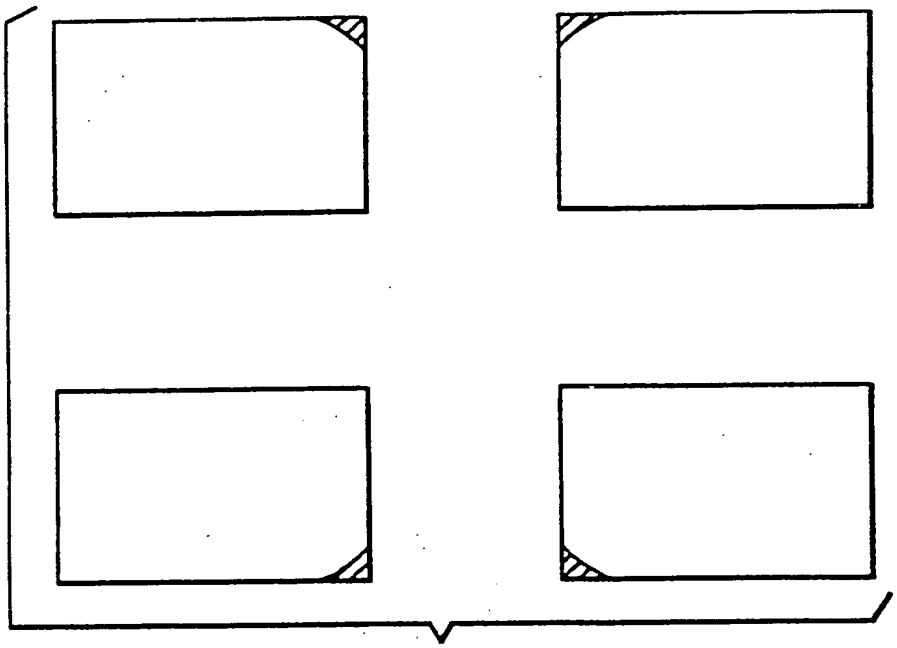

Fig. 23

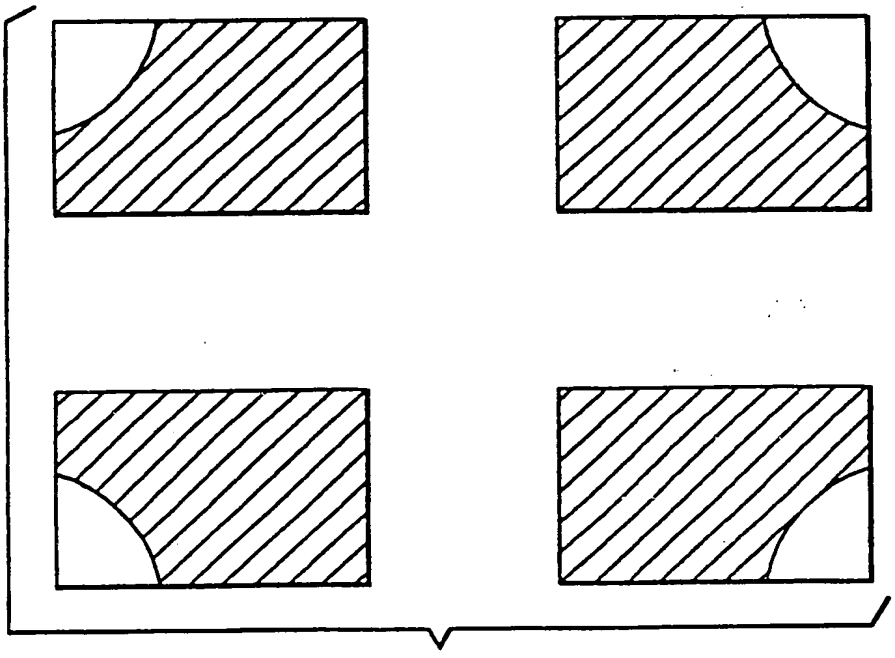

Fig. 24

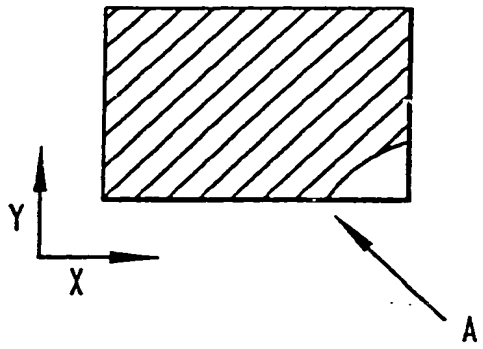

Fig. 25

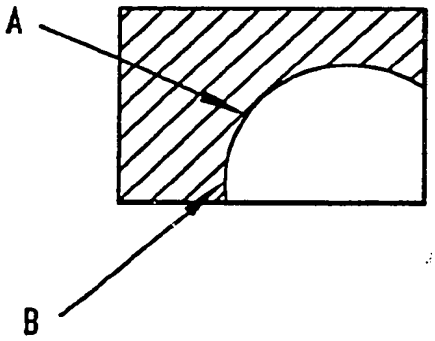

Fig. 26

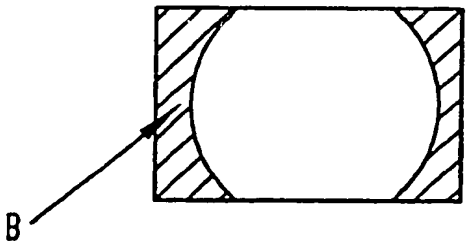

Fig. 27

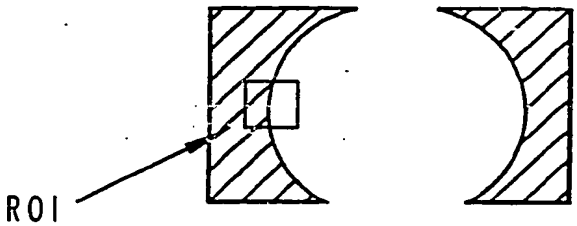

Fig. 28

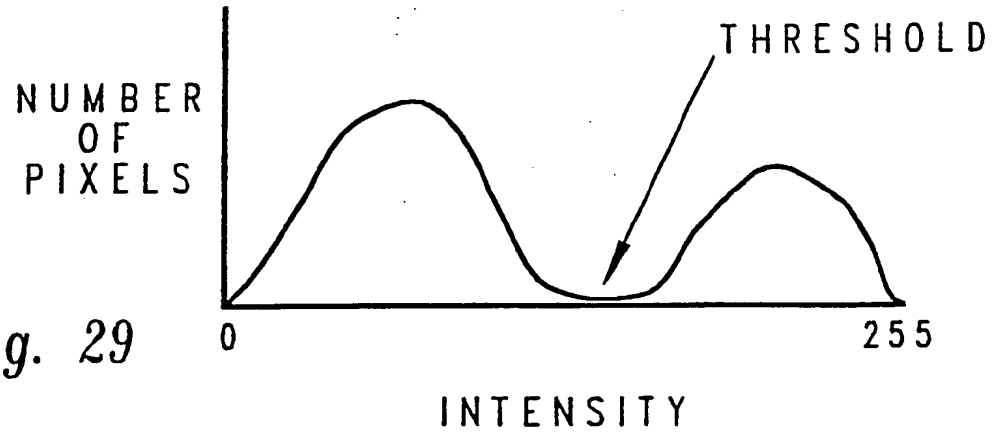

Fig. 29

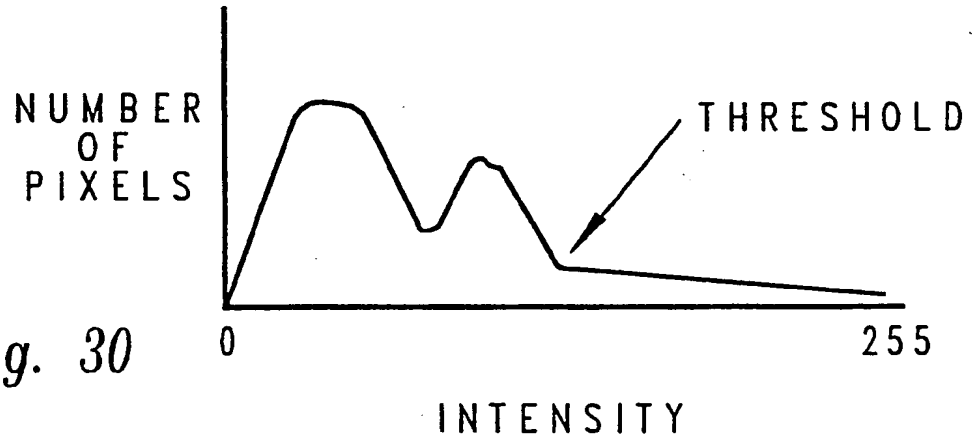

Fig. 30

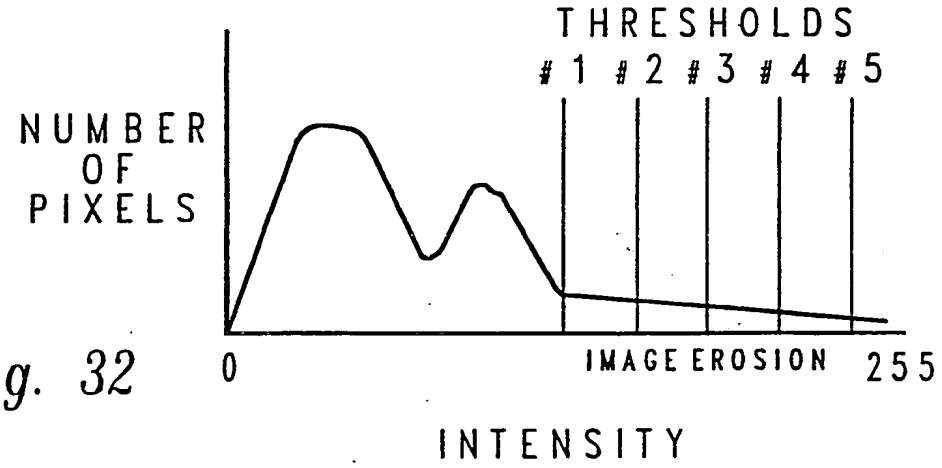

Fig. 32

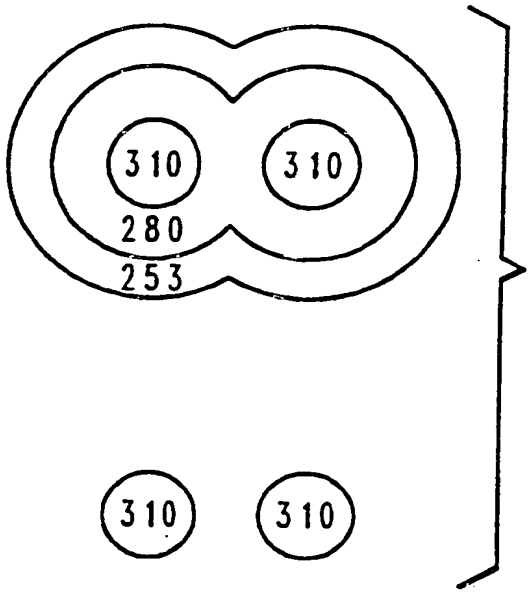

Fig. 31

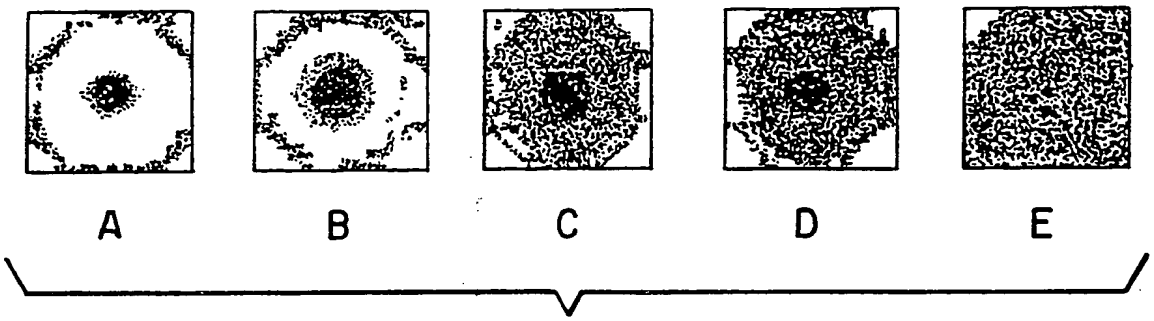

FIG. 33

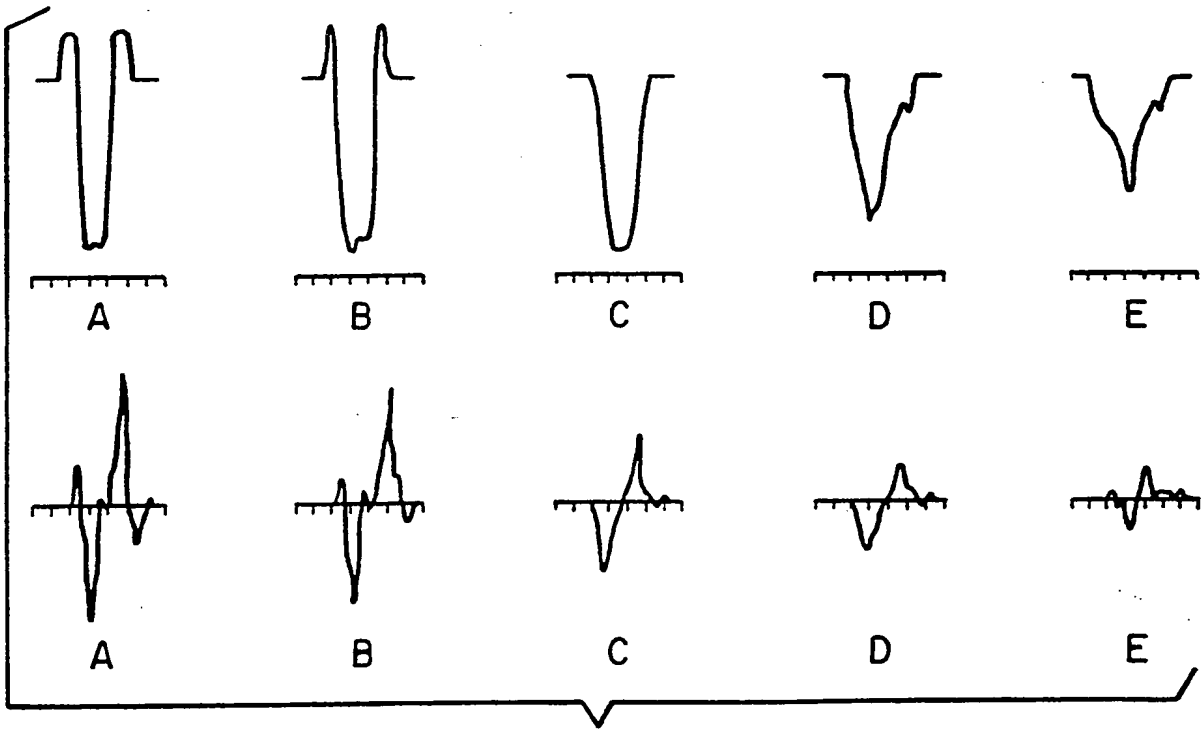

FIG. 34

INTERNATIONAL SEARCH REPORT

Inter. ional application No.
PCT/US92/05015

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 21/01, 35/06; B01L 3/02
US CL :73/304C, 864.24, 864.25; 422/ 67, 100, 106; 436/43, 54
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 73/290R, 304C, 864.22, 864.23, 864.24, 864.25; 422/63, 65, 67, 100, 102, 106; 436/43, 47, 48, 54, 526

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, search terms: HLA, fluid, liquid, level, surface, detect###, rf, radio frequency, interface

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO, A, 91/16675 (Cathcart et al.) 31 October 1991, see entire document and fig. 9(A-D) specifically.	1-10
Y	US, A, 4,326,851 (Bello et al.) 27 April 1982, see entire document.	1-6
Y	US, A, 4,736,638 (Okawa et al.) 12 April 1988, see entire document.	1-6
Y	US, A, 4,818,492 (Shimizu) 04 April 1989, see entire document.	1-6
Y	US, A, 4,897,244 (Wallace et al.) 30 January 1990, see entire document.	1-6
Y	US, A, 4,912,976 (Labriola, II) 03 April 1990, see entire document.	1-6
Y	US, A, 4,939,925 (Sakuma et al.) 10 July 1990, see entire document.	1-6
Y	US, A, 4,970,468 (Ishizawa et al.) 13 November 1990, see entire document.	1-6
Y,P	US, A, 5,027,075 (Harding, Jr.) 25 June 1991, see entire document.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 SEPTEMBER 1992	Date of mailing of the international search report 18 SEP 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ARLEN SODERQUIST Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inte..ational application No.
PCT/US92/05015

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US, A, 5,049,826 (Sasao) 17 September 1991, see entire document.	1-6
Y	US, A, 4,586,546 (Mezei et al.) 06 May 1986, see figures 1, 3, 4, and the associated discussion.	7-9
A	Clinical Chemistry, Vol 34, issued 1988, M. Flore et al., "The Abbott IMx™ Automated Benchtop Immunochemistry Analyzer System", pages 1726-1732.	1-17
A	US, A, 4,228,831 (Kerns) 21 October 1980.	1-6
A	US, A, 4,659,677 (Glover et al.) 21 April 1987.	7-10
A	US, A, 4,869,114 (Kido et al.) 26 September 1989.	7-10